(11)Publication number : **10-146183**
(43)Date of publication of application : **02.06.1998**

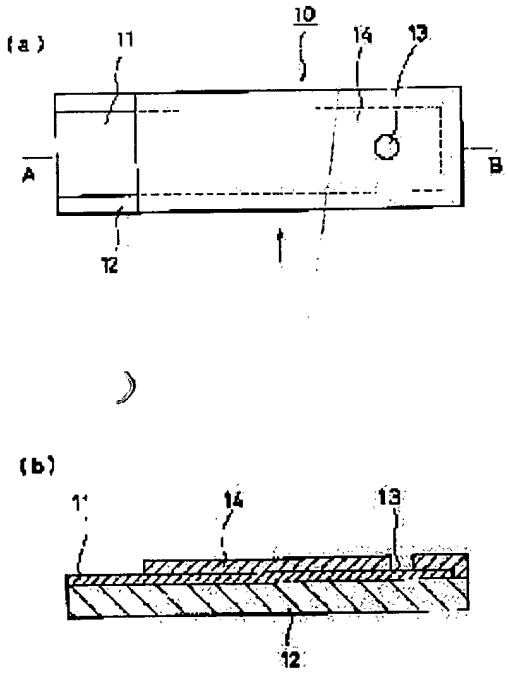
(51)Int.CI. C12M 1/00
// C12N 15/09
C12Q 1/68

(21)Application number : **09-032413**
(22)Date of filing : **17.02.1997**

(71)Applicant : **TOSHIBA CORP**
(72)Inventor : **HASHIMOTO KOJI**
ITO KEIKO
MISHIRO TOSHIHARU
ISHIMORI YOSHIO

(30)Priority
Priority number : **08248333** Priority date : **19.09.1996** Priority country : **JP**

(54) ELECTRODE, DETECTION DEVICE AND SENSOR



(57)Abstract:
PROBLEM TO BE SOLVED: To obtain an electrode comprising a substrate, a conductor disposed on the substrate, an insulator coated on the conductor, while securing a connection region on the surface of the conductor to an outer member, the opening of the insulator, and a nucleic acid immobilized on the exposed conductor, and useful for a sensor capable of detecting a nucleic acid.
SOLUTION: This electrode 10 useful for a sensor for detecting a target nucleic acid, etc., comprises a substrate 12, a conductor 11 disposed on the substrate 12, an insulator 14 coated on the surface of the conductor 11, while securing a connection region to an outer member, an opening 13 disposed in the insulator so as to partially expose the conductor 11, and the first nucleic acid immobilized on the conductor 11 exposed in the opening 13. The detection of the target nucleic acid is carried out by hybridizing the first nucleic acid immobilized on the electrode 10 with the second complementary nucleic acid on the electrode in preset conditions, applying a voltage to the first nucleic acid immobilized on the electrode and subsequently measuring the signal generated by the application.

LEGAL STATUS

[Date of request for examination]	14.03.2000
[Date of sending the examiner's decision of rejection]	03.12.2002
[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]	
[Date of final disposal for application]	
[Patent number]	
[Date of registration]	
[Number of appeal against examiner's decision of rejection]	2003-00275
[Date of requesting appeal against examiner's decision of rejection]	06.01.2003
[Date of extinction of right]	

TECHNICAL FIELD

[The technical field to which invention belongs] this invention relates to the sensor using the electrode which fixed the detection equipment and the nucleic acid using the electrode which fixed the nucleic acid, and the electrode which fixed the nucleic acid.

PRIOR ART

[Description of the Prior Art] By progress of molecular biology in recent years, the relation between the base sequence of a nucleic acid and the illness is becoming clear. Then, the gene diagnosis which explores the possibility of the onset of a disease, the grade of advance of the illness, etc. has come to occupy an important position in a clinical test by detecting existence of a specific base sequence or a specific gene.

[0003] Generally the electrode which detects existence of a specific base sequence or a specific gene has taken on the conductor the composition which fixed the nucleic acid used as a probe, and existence of a specific base sequence or a specific gene is detected by measuring an electric or optical signal for the hybrid formed between the nucleic acid used as a probe, and the nucleic acid in a sample. However, in order that the conventional electrode may take the structure which ****(ed) the wire-like electrode by the resin, the surface area of the field which fixes an electrode and the nucleic acid which serves as a probe in a conductor especially tends to vary. Furthermore, the crystal structure of the matter is difficult to be unable to control the crystal structure by the electrode which has the form of the shape of a conventional wire, but to control the amount of the probe fixed on a conductor, although affecting the amount of other matter fixed by the front face is known. Therefore, it was tended to change the amount of the probe

fixed on a conductor, and there was a problem that repeatability was bad and inferior also to fixed quantity nature on the occasion of detection of a nucleic acid.
[0004] moreover, a viewpoint to 105 which high sensitivity-ization of detection sensitivity is advancing in the field of gene diagnosis in recent years, for example, is used for the index of medical treatment or the advance state of the illness in the gene diagnosis of a hepatitis C virus - a 106 copy/mL grade -- until -- the sensitivity which can detect a nucleic acid is demanded Similarly, high detection sensitivity is called for also in viruses, such as HIV and HBV, a bacterial inspection or detection of an oncogene, etc., and the further high sensitivity-ization is called for. Furthermore, in order to mitigate the burden to a patient, minute amount-ization of the sample to extract is advancing and measurement has already been performed from the sample of several micro liter in blood electrolyte measurement.
[0005] However, by the conventional detection equipment and the conventional sensor using the electrode, since the combination to the probe of an intercalating agent or a label-ized agent cannot be prevented when the matter which has electrochemical activity carries out a direct reaction on the surface of an electrode or the label-ized agent which has an intercalating agent and electrochemical activity is used, a background will become large. Therefore, abundance is the limitation of detection of the nucleic acid to a 105 copy/mL grade (3830 KHashimoto, Anal.Chem.66, 1994), and there was a problem that detection sensitivity was low, like the PCR method as compared with the method of detection of the nucleic acid using the enzyme reaction.
[0006] Furthermore, according to detection equipment, a sensor, etc. which applied the method of detecting a nucleic acid using the label-ized agent which has the method or intercalating agent which combined rho CR process mentioned above, the nucleic-acid probe which carried out enzyme labeling, and the chemiluminescence, and electrochemical activity, since the system of reaction was complicated, the time which detection of a nucleic acid takes is long, operation is complicated, and the economical burden which detection of a nucleic acid takes also had the problem were large.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] The schematic diagram of the electrode concerning one operation gestalt of this invention.
[Drawing 2] The schematic diagram of the electrode concerning one operation gestalt of this invention.
[Drawing 3] The schematic diagram of the electrode concerning another operation gestalt of this invention.
[Drawing 4] The schematic diagram of the electrode concerning another operation gestalt of this invention.
[Drawing 5] still more nearly another operative condition of this invention -- the schematic diagram of the electrode built like
[Drawing 6] Drawing having shown one embodiment of the detection equipment using the electrode concerning this invention.
[Drawing 7] Drawing of the detection equipment completely automated using the electrode

concerning this invention having shown composition [like] 1 operative condition.
[Drawing 8] Drawing of the detection equipment completely automated using the electrode concerning this invention having shown composition [like] 1 operative condition.
[Drawing 9] Drawing having shown one embodiment of the sensor concerning this invention.
[Drawing 10] Drawing having shown one embodiment of the sensor concerning this invention.
[Drawing 11] Drawing having shown one embodiment of the sensor concerning this invention.
[Drawing 12] Drawing having shown one embodiment of the electric composition in the detection equipment using the sensor concerning this invention.
[Drawing 13] Drawing having shown typically the detection equipment using the sensor concerning this invention.
[Drawing 14] Drawing having shown typically the detection equipment using the sensor concerning this invention.
[Drawing 15] Drawing having shown one embodiment of the detection equipment using the sensor concerning this invention.
[Drawing 16] Drawing having shown the limit of detection of hepatitis B virus-DNA in an example 23.
[Description of Notations]
10, 20, 30, 40, 50, 61,131,132 .... Electrode
11, 93, 94,104,105,106 .... Conductor
12, 35, 54, 91, 92 .... Substrate
101, 102, 103, 121, 122 .... Substrate
13, 31, 32, 51 .... Opening
14, 33, 53 .... Insulator 15 .. Glue line
34 55 .... Lead wire 52 .. Reference electrode
62, 95, 96,107,108,109,110 .... Nucleic acid
63 .... Reaction vessel 64,125 .. Power supply
65,126,135 .... Ammeter 66,127,136 .. Voltmeter
67,128,137 .... Variable resistance 68 .. Counter electrode
70 80,150 .... Detection equipment 71 .. Electrode fixed electrode holder
72 .... Reaction vessel 73 .. The 1st washing tub 74 .. Intercalating-agent solution tub
74 .... Intercalating-agent solution tub 75 .. The 2nd washing tub 76 .. Electrochemistry measurement tub
77 .... Move equipment 78 .. Analysis unit 79 .. Operation unit
81 .... Sample carrying-in mouth 82 .. Sample conveyance unit
83 .... Sample preparation unit 84 .. Control unit
85 .... Waste storage unit 86 .. Waste fluid storage unit
87 .... Reagent supply unit 88 .. Analysis unit 112 .. Spacer
133 .... Capillary tube 141 158 .. Liquid-sending pump
142 153 .... Pouring section 143 .. Measuring device
144 152 .... Solution tank 145 154 .. Waste fluid tank
151 .... Fixed electrode holder 155 .. Liquid-sending line 156 .. Measurement unit
157 .... Output terminal

DETAILED DESCRIPTION

[Problem(s) to be Solved by the Invention] this invention was made in view of the above-mentioned conventional example, demonstrates the outstanding repeatability and outstanding fixed quantity nature, and aims at offering economically the electrode which can detect a nucleic acid.
[0008] Moreover, this invention aims at offering the detection equipment which is excellent also in the economical efficiency at the time of the detection by which shortening of time and the improvement in operability which detection takes were achieved while it demonstrates the outstanding repeatability and outstanding fixed quantity nature on the occasion of detection of a nucleic acid.
[0009] Furthermore, high sensitivity-ization of detection sensitivity is attained and this invention aims at offering the detection equipment which is excellent also in the economical efficiency at the time of the detection by which shortening of time and the improvement in operability which detection takes were achieved while it demonstrates the outstanding repeatability and outstanding fixed quantity nature on the occasion of detection of a nucleic acid.
[0010] Moreover, high sensitivity-ization of detection sensitivity is attained and this invention aims at offering the sensor which is excellent also in the economical efficiency at the time of the detection by which shortening of time and the improvement in operability which detection takes were achieved while it demonstrates the outstanding repeatability and outstanding fixed quantity nature on the occasion of detection of a nucleic acid.
[0011]
[Means for Solving the Problem] The electrode concerning this application the 1st invention is characterized by providing a substrate, the conductor arranged on the aforementioned substrate, the insulator covered securing a connection field [as opposed to the exterior for the front face of the aforementioned conductor], opening prepared in the aforementioned insulator so that the aforementioned conductor might be exposed, and the nucleic acid fixed in the aforementioned conductor exposed from the aforementioned opening.
[0012] Since a nucleic acid is fixable to up to the conductor by which the surface area was controlled by having prepared opening so that a conductor might be exposed to some insulators which covered the conductor arranged on a substrate, and having fixed the nucleic acid in the conductor exposed from this opening according to the electrode in this application the 1st invention, it becomes possible to control the amount of the nucleic acid to fix. Moreover, since the property of a conductor is controllable by having arranged the conductor on a substrate, it becomes possible to control the amount of the nucleic acid fixed on a conductor.
[0013] In the electrode concerning this application the 1st invention, especially the quality of the material of a substrate is not limited, and can use an inorganic insulating material, an organic material, etc. As an inorganic insulating material, the metallic oxide of glass, quartz glass, an alumina, sapphire, a forsterite, silicon carbide, silicon oxide, silicon nitride, and others etc. can be mentioned. As an organic material, moreover, polyethylene, ethylene, a polypropylene bilene, A polyisobutylene, a polyethylene terephthalate, a unsaturated polyester, A fluorine-containing resin, a polyvinyl chloride, a polyvinylidene chloride, polyvinyl acetate, Polyvinyl alcohol, a polyvinyl acetal, acrylic resin, a polyacrylonitrile, Polystyrene, an acetal resin, a polycarbonate, a polyamide, phenol resin, A urea resin, an epoxy resin, melamine resin, a styrene acrylonitrile copolymer, acrylonitrile-butadiene-styrene copolymer, silicone resin, a polyphenylene oxide, a polysulfone, etc. can be mentioned.
[0014] Moreover, as the quality of the material of the conductor arranged on a substrate, although gold is desirable, the other quality of the materials are also usable. For example, carbon,

and these oxide or compounds, such as metal simple substances, such as a golden alloy, silver, platinum, mercury, nickel, palladium, silicon, germanium, a gallium, and a tungsten, or those alloys, graphite, and GURASHI carbon, can be mentioned.
[0015] Furthermore, arrangement of the conductor to a substrate top can be performed by methods, such as vacuum evaporationo, a spatter, plating, and printing. As a vacuum deposition, a resistance heating method, a high-frequency-heating method, an electron-beam-heating method, etc. can be mentioned. Moreover, as a spatter, direct-current two-poles sputtering, bias sputtering, unsymmetrical alternating current sputtering, getter sputtering, RF sputtering, etc. can be mentioned.
[0016] Moreover, in the conductor arranged to up to a substrate, it becomes it is more desirable and possible [controlling the amount of the nucleic acid fixed on a conductor by this] to enlarge the orientation index of the field (111) of the crystal structure. In addition, an orientation index is called for from the following formulas by the method of Wilson.
[0017] Orientation index (h k l) = IF (hkl)/IFR (hkl)
Here, the relative intensity of a field (h k l) and iotaFR (hkl) are IF (hkl) as a standard gold hkl is indicated by indices of crystal plane and IF (hkl) is indicated to be by the ASTM card.
[0018] In the electrode concerning this application the 1st invention, it is desirable that the above-mentioned orientation index is three or less [1 or more], and it is more desirable that it is [or more 2] three or less. In order to make the orientation index of a conductor high, it is effective to heat a substrate when arranging a conductor on a substrate by vacuum evaporationo or sputtering. Although especially heating temperature is not limited, it is desirable that it is [50 degrees-C or more] 500 degrees C or less.
[0019] Moreover, in case a conductor is arranged on a substrate, the glue line which consists of the different quality of the material from the quality of the material used for this conductor between this substrate and this conductor is made to intervene, and if it constitutes so that a conductor may get used to a glue line, it will become possible to raise the stability of a conductor. As the quality of the material of a glue line, what combined the alloy and these simple substances, or alloy of titanium, chromium, copper, the simple substance of nickel, and these simple substances can be mentioned. Also in this case, in case a conductor is arranged on a glue line by vacuum evaporationo or sputtering, it is effective to heat a glue line.
[0020] furthermore -- as the quality of the material of an insulator -- desirable -- a resin -- a photopolymer and a photoresist can be mentioned more preferably As a photoresist, the photoresist for optical exposure, the photoresist for far-ultraviolet, the photoresist for X-rays, and the photoresist for electron rays can be mentioned. As a photoresist for optical exposure, what uses cyclized rubber, the poly **** leather acid, a novolak resin, etc. as the main raw material can be mentioned. As a photoresist for far-ultraviolet, what uses cyclized rubber, phenol resin, the poly methyl isopropenyl ketone (rhoMiotaPK), a polymethylmethacrylate (rhoMMalpha), etc. as the main raw material can be mentioned. Moreover, as a resist for X-rays, although COrho and metal acrylate are used as the main raw material, the thing which was indicated by the others and thin film handbook (Ohm-Sha Ltd.) and which is known for the technical field concerned can be mentioned. Furthermore, as a resist for electron rays, what was indicated by the above-mentioned handbooks including rhoMMalpha can be mentioned. In addition, because of insulating reservation, the thickness of an insulator has desirable 100A or more, and it is desirable that it is 1mm or less because of fixation of a nucleic acid.
[0021] Furthermore, opening prepared in the insulator so that a conductor might be exposed can be formed using the lithography known for the technical field concerned, after covering a

conductor with an insulator. Therefore, it becomes possible to control the size of opening, and the surface area of the conductor exposed from the insulator when putting in another way, and the amount of the fixed nucleic acid can be controlled. In addition, as for the configuration of the front face of the conductor exposed from the insulator, it is desirable that it is a polygon more than the configuration which curvature change follows, for example, a circle, an ellipse, or a right hexagon.

[0022] Facing performing lithography, one of the desirable modes of an insulator is the resist mentioned above. Although removing finally is common conventionally as for a resist, in the case of the electrode which is this application the 1st invention, it must be possible to use for a part of electrode as an insulator, and it must be the waterproof high quality of the material mentioned above. Moreover, silicon besides a resist, titanium, aluminum, zinc, lead, cadmium, Oxides, such as a tungsten, molybdenum, chromium, a tantalum, and nickel, After forming a thin film by methods, such as a spatter, vacuum evaporationo, or CVD, using a nitride and carbide, or other alloys Patterning of opening can be performed by performing lithography, such as photo lithography, and the size of opening and the surface area of the conductor exposed from the insulator when putting in another way can be controlled.

[0023] Moreover, when it fixes a nucleic acid to the conductor exposed to opening from the insulator, it is desirable to perform processing which activates the front face of a conductor. By removing the foreign matter which exists on the surface of an insulator, and exposing the front face of an insulator completely, this processing makes the free energy in the front face of an insulator increase, and it is performed in order to ensure fixation of a nucleic acid. Specifically, a conductor is first washed by deionized water. After washing and in the inside of the sulfuric-acid solution of 0.1-10M - Potential is made to scan in the range of 1 - 100000 v/s in 0.5-2V (vs Ag/AgCl). In addition, you may perform activation not only using the solution of a sulfuric acid but using a mixed acid, an aqua regia, perchloric acid, etc. In this way, although a nucleic acid is fixed in the conductor by which the front face was activated, the thiol group is introduced into 5' of a nucleic acid, or 3'end on the occasion of fixation. The thiol-ized nucleic acid is saved in the solution with which reducing agents, such as DTT, exist from a viewpoint which mainly prevents oxidization of a thiol group. Reducing agents, such as DTT, are removed by the extract operation by the gel filtration or ethyl acetate etc. just before fixation of a nucleic acid. In order to fix the nucleic acid which introduced the thiol group in the conductor by which the front face was activated, the electrode immediately after ionic strength's dissolving a nucleic acid into the buffer solution with which 0.01-5, and pH were adjusted within the limits of 5-10, and activating it to this buffer solution is performed by being immersed. Fixation of the nucleic acid to a conductor makes temperature of the buffer solution the range of 4-100 degrees C, from 10 minutes, is left one evening and performed. Since a thiol group is adsorbed on the front face of conductors, such as gold, and it is fixed by the strong interaction, the nucleic acid which introduced the thiol group will be firmly fixed on the surface of a conductor. Although controlled by adjusting the amount of the nucleic acid dissolved in the buffer solution mentioned above, generally the amount of the nucleic acid fixed by the conductor is adjusted so that the last concentration of the nucleic acid in the buffer solution may become in ml and 1 ng/ml - 1mg /. Under this condition, a nucleic acid is 10 9 copy/cm2 - 1014 copy/cm2 to the front face of a conductor. Grade fixation is carried out. The electrode which fixed the nucleic acid, and DNase It can save on the conditions in which nucleases (nuclease), such as RNase, do not exist, and can save by shading preferably. When a retention period is comparatively short, it is possible to dip and save in a hybridization solution, the tris-EDtauA buffer solution (TE), or the deionized water that sterilized. Although the storage

temperature of an electrode has desirable 4 degrees C or less, it saves at about -20 degrees C more preferably. In addition, when saving an electrode over a long period of time, in order to keep stable the fixed nucleic acid, saving under dry conditions is desirable. As a method of making an electrode dry, freeze drying, air-drying, etc. can be mentioned and it can carry out suitably under gaseous phases, such as inert gas, such as an argon, nitrogen, or dry air, or a vacua.

[0024] Furthermore, RNA (single strand) besides DNA (single strand) is sufficient as the nucleic acid fixed on the surface of a conductor, and the number of bases is not limited, either. However, in order to detect a specific nucleic acid certainly, without producing the background, as for a nucleic acid, it is desirable to make it about [15 base -3000 bases] length. Moreover, although the base sequence of the nucleic acid fixed on the surface of a conductor is determined according to the base sequence of the nucleic acid to detect, the nucleic acid which consists not only of one kind of base sequence but of two or more kinds of base sequences is fixable on the surface of a conductor. You may be a nucleic acid originating in the cell which has various disorders including the nucleic acid and tumor which originate in various viruses, bacteria, parasites, fungi, etc. as a nucleic acid to detect. In addition, the nucleic acids to detect may be a part of gene and gene originating in the cell which has various disorders including various virus, bacteria, parasites, fungi, and tumors, and may be nucleic acids other than a gene. Here, a gene points out a specific partition (structural gene) with a certain length on a genome. As a virus, a hepatitis virus (A, beta, C, D, E, F, and G type), human immunodeficiency virus, influenza viruses, the Herpesvirus, adenovirus, the polyoma virus, a papilloma virus, a parvovirus, a mumps virus, the rotavirus, an enterovirus, a Japanese encephalitis virus, dengue virus, the rubella virus, a Homo sapiens tau cell leukosis virus, a cytomegalovirus, etc. can be mentioned. Moreover, as bacteria and a fungus, Staphylococcus aureus, a hemolytic streptococcus, an E. coli bacillus, a vibrio-parahemolytica bacillus, a HERIKOBAKUTABIRORI bacillus, can BIROBAKUTA, a cholera vibrio, a dysentery bacillus, a salmonella, a Yersinia, Neisseria gonorrhoeae, Listeria monocytogenes, a rep toss villa, legionella bacteria, spirohete, mycoplasma pneumonia, RIKKECHIA, air conditioner JIMIA, malaria, an Entamoeba histolytica, a pathogenic fungus, etc. can be mentioned. Moreover, as a cell which has various disorders, the cell originating in a retinoblastoma, a Wilms' tumor, the familial large intestine polyposis, hereditary polyposis colon cancer, neurofibromatosis, a familial breast cancer, a xeroderma pigmentosum, a brain tumor, cancer of the mouth, an esophagus cancer, stomach cancer, colon cancer, liver cancer, a pancreatic cancer, lung cancer, a thyroid tumor, a urinary-organs tumor, a virilia tumor, a female machine tumor, the ecphyma, a bone and a soft-parts tumor, leukemia, a lymphoma, and a solid tumor can be mentioned. Furthermore, the nucleic acid fixed on the surface of a conductor may be extracted from samples, such as blood, a blood serum, a leucocyte, urine, facilities, sperm, saliva, an organization, a cultured cell, and expectoration, and may carry out chemosynthesis with a DNA synthesis machine etc. It is also possible for it not to be limited, and to be able to use solvent extraction, such as the phenol-chloroform method, and the solid-liquid extraction method using support especially as a method of extracting a nucleic acid from the above-mentioned sample, and to use the nucleic-acid extraction kit of marketing, such as QIAmp (product made from QIAGEN) and a SUMAI test (Sumitomo Metal Industries, Ltd. make).

[0025] If detection of a nucleic acid is required for the electrode by this application the 1st invention, it is applicable to all fields, such as not only the medical field that detects various disorders and the grade of advance of the illness but food evaluation, quarantine, drug inspection, the medical jurisprudence, agriculture, a zootechnics, a fishing, forestry, etc.

[0026] Moreover, the conductor which has arranged the detection equipment concerning this application the 2nd invention on a substrate and the aforementioned substrate, The insulator covered securing a connection field [as opposed to the exterior for the front face of the aforementioned conductor], The electrode which has opening prepared in the aforementioned insulator so that the aforementioned conductor might be exposed, and the 1st nucleic acid fixed in the aforementioned conductor exposed from the aforementioned opening, The reaction section which performs hybridization under the conditions which the 1st nucleic acid and 2nd nucleic acid which were fixed by the aforementioned electrode were made to live together, and were set up, It is characterized by providing an impression means to impress voltage to the 1st nucleic acid fixed by the aforementioned electrode, and a measurement means to measure the signal produced by operation of the aforementioned impression means.
[0027] By according to the detection equipment in this application the 2nd invention, carrying out hybridization of the 1st nucleic acid and 2nd nucleic acid which were fixed quantitatively to an electrode in the reaction section, and measuring the signal which impressed voltage to the 1st nucleic acid and was produced Since the measurand of this signal and the amount of the 2nd nucleic acid can be made to correspond uniquely, while excelling in repeatability and fixed quantity nature, it becomes possible to detect the 2nd nucleic acid by high sensitivity. Moreover, since shortening of time and the improvement in operability which detection takes are attained, it becomes possible to detect the 2nd nucleic acid economically.
[0028] In the detection equipment concerning this application the 2nd invention, an electrode is equivalent to the electrode concerning this application the 1st invention mentioned above.
[0029] Moreover, if the reaction section makes the 1st nucleic acid and 2nd nucleic acid which were fixed by the electrode live together and the composition which can perform hybridization is taken Although not limited especially, what has the structure in which temperature management is possible is desirable while it prevents evaporation of the buffer solution, since about one evening of hybridization is made to react 1 minute or more at the temperature of 10-90 degrees C using the buffer solution of ionic strength 0.01-5 and the range of peta5-10 generally.
[0030] Furthermore, as an impression means to impress voltage to the 1st nucleic acid fixed by the electrode, the field which fixed the 1st nucleic acid of this electrode is made into an operation pole, a matched-pairs pole is established in this operation pole, and limitation will not be carried out if it is constituted so that voltage may be impressed between an operation pole and a counter electrode. Moreover, you may install the reference electrode which makes the gland other than an operation pole and a counter electrode the frame of reference. Moreover, usually, the operation pole and the counter electrode are arranged through the liquid, and if this liquid does not bar the impression of voltage to the 1st nucleic acid, and the measurement mentioned later excluding the nuclease which decomposes a nucleic acid, limitation will not be carried out.
[0031] Moreover, a means to detect electric signals, such as current value, electrical conductivity, potential, resistance, electric capacity, an inductance, and an impedance, as a measurement means to measure the signal produced by operation of an impression means, and a means to detect fluorescence, a chemiluminescence, and an electrogenerated chemiluminesence are mentioned. Since a nucleic acid will emit light from self if voltage is impressed, it can detect the amount of the nucleic acid which exists in an electrode, and the amount of the 2nd nucleic acid which carried out hybridization to the 1st nucleic acid when putting in another way by measuring luminescence intensity. In order to acquire an electric signal on the occasion of detection of the 2nd nucleic acid, moreover, the HOECHST 33258, an acridine orange, Screw intercalators, such as a quinacrine, DOUNO mycin, a METARO intercalator, and a screw

acridine, Intercalating agents, such as a tris intercalator and the poly intercalator, Carry out the indicator of the nucleic acid on an electrode with the activity matter electrochemically [FIROSEN, a metal complex, etc.], and electric signals, such as a reaction current value which originates in the matter [activity / this electrochemistry target] by impressing voltage to the 1st nucleic acid, are detected. The amount of the 1st nucleic acid and the 2nd nucleic acid which carried out hybridization is detectable. Electric measurement is performed by 3 electrode type (a reference electrode, a counter electrode, operation pole) or 2 electrode type (a counter electrode, operation pole), voltage is impressed so that an intercalating agent etc. may react electrically, and the current value originating in an intercalating agent etc. is measured. Under the present circumstances, voltage can be swept by fixed speed, can be impressed by the pulse, or can impress a constant voltage. In measurement, current and voltage are controlled using equipments, such as a potentiostat, a multimeter, and a function generator. And the concentration of the 2nd nucleic acid is computed from a calibration curve based on the obtained current value. Moreover, on the occasion of detection of the 2nd nucleic acid, in order to amplify an optical signal, you may add a photogene and a fluorescent substance. As a fluorescent substance, intercalating agents, such as screw intercalators, such as the HOECHST 33258, an acridine orange, a quinacrine, DOUNO mycin, a METARO intercalator, and a screw acridine, a tris intercalator, and the poly intercalator, can be mentioned. These intercalating agents emit fluorescence by irradiation of ultraviolet rays etc. Moreover, the indicator of the 2nd nucleic acid is carried out by photogenes, such as fluorescent substances, such as a rhodamine and fluorescein, and luciferin and acridinium ester, a photon is generated by adding irradiation, an enzyme, etc. of ultraviolet rays etc., and the amount of the 1st nucleic acid and the 2nd nucleic acid which carried out hybridization can be detected. Furthermore, the amount of the 1st nucleic acid and the 2nd nucleic acid which carried out hybridization is detectable by carrying out the indicator of the 2nd nucleic acid, and making it emit light by electrogenerated-chemiluminesence matter, such as lucigenin and a ruthenium complex. Moreover, the amount of the 1st nucleic acid and the 2nd nucleic acid which carried out hybridization is detectable using alkaline phosphatase, a par oxidase, etc. Moreover, although the concentration of an intercalating agent, a photogene, the electrogenerated-chemiluminesence matter, a fluorescent substance, and an enzyme changes with the kinds on the occasion of an indicator, it is used for - ** in the 1 ng/ml - 1mg [/ml] range. As for an indicator, at this time, it is good to carry out using the buffer solution of ionic strength 0.01-5 and the range of pH 5 to 10. therefore, a measurement means has the optimal independent thing if needed -- or it is put together and used In addition, in order to suppress the non-specific adsorption of indicator agents, such as an intercalating agent, a photogene, electrochemistry, a photogene, and a fluorescent substance, it is desirable to cover with a certain matter the front face of the field which fixes the 1st nucleic acid at the time after fixation of the 1st nucleic acid of fixation. Although especially this matter is not the object limited, when a sensor consists of gold, it is more desirable that they are thiol compounds. A thiol-ized nucleic acid can be used as thiol compounds. There are a nucleotide, a deoxy nucleotide, a nucleoside, and a guanine deoxyriboside in a thiol-ized nucleic acid, and an adenine, a thymine, a hypoxanthine, a xanthin, a cytosine, a guanine, a uracil, an inosine, an adenosine, thymidine, a guanosine, a cytidine, a uridine and ribothymidine, or these oligomer can be mentioned. Moreover, thiol compounds, such as dithiothreitol, a dithio screw benzene benzoic acid, and an alkane thiol mercaptoethanol, can also be used. When the front face of the field which fixes the 1st nucleic acid is covered with this matter, un-unique adsorption of the indicator agent to this field will be suppressed, and S/N will improve. Moreover, when measuring the signal which the

impression means was operated and was produced by the measurement means, it is desirable to wash the electrode after hybridization by the hybridization solution, the sterilized water, etc. from the purpose which reduces the background.

[0032] Furthermore, the electrode to which the detection equipment concerning this application the 3rd invention fixed the 1st nucleic acid in the field which has the minimum area required for detection of the 2nd nucleic acid, The reaction section which performs hybridization under the conditions which the 1st nucleic acid and 2nd nucleic acid of the above which were fixed by the aforementioned electrode were made to live together, and were set up, It is characterized by providing an impression means to impress voltage to the 1st nucleic acid fixed by the aforementioned electrode, and a measurement means to measure the signal produced by operation of the aforementioned impression means.

[0033] According to the detection equipment in this application the 3rd invention, hybridization of the 1st nucleic acid and 2nd nucleic acid which were fixed quantitatively is carried out in the reaction section into the field which has the minimum area required for detection of the 2nd nucleic acid. Since the background can be reduced while making the measurand of this signal, and the amount of the 2nd nucleic acid correspond uniquely by measuring the signal which impressed voltage to the 1st nucleic acid and was produced While excelling in repeatability and fixed quantity nature, it becomes possible to detect the 2nd nucleic acid by high sensitivity. Moreover, since shortening of time and the improvement in operability which detection takes are attained, it becomes possible to detect the 2nd nucleic acid economically.

[0034] As mentioned above, also at the lowest by inspection of hepatitis C virus, the sensitivity of 105 copy/mL is demanded of the index of treatment now. Moreover, detection of inspection of HIV, hepatitis B virus, etc. or the microbism, an oncogene, etc. being also equivalent or the high detection sensitivity beyond it is called for. Generally, with the detection equipment which detects a nucleic acid and performs hybridization, an activity marker etc. will carry out a direct reaction on the surface of an electrode optically and electrochemically, or a background will become large because an activity marker etc. combines [an intercalating agent and] with an intercalating agent or the nucleic acid (probe) fixed by the electrode optically and electrochemically. Therefore, it is the cause of reducing the detection sensitivity of a nucleic acid with an un-unique signal (background). In order to raise the detection sensitivity of a nucleic acid, it is important to measure only the specific signal which is made to reduce an un-unique signal (background) and originates in hybrid formation. That is, if only the specific signal originating in the hybrid formed on the electrode is certainly detectable, high sensitivity-ization is expectable about detection of a nucleic acid. In order to reduce the un-unique signal mentioned above, optically and electrochemically, an activity marker etc. carries out a direct reaction on the surface of an electrode, or should just suppress optically and electrochemically an intercalating agent and that an activity marker etc. combines with an intercalating agent or the nucleic acid (probe) fixed by the electrode. And in order to attain this suppression, it becomes effective to make small area of the field which fixes a nucleic acid. Namely, by making small area of the field which fixes a nucleic acid, while an activity marker etc. decreases an intercalating agent and the amount combined with the front face of an electrode, and the field which fixes a nucleic acid especially in un-unique optically and electrochemically the intercalating agent combined with a nucleic acid in un-unique as a result since the amount of the nucleic acid fixed by the electrode also decreases -- amounts, such as an activity marker, will also decrease optically and electrochemically Therefore, the un-unique signal originating in an intercalating agent etc. can be suppressed only by making small area of the field which fixes a nucleic acid. Here, being shown

by the formula (1) made clear the relation with the area of the field which fixes the detection sensitivity and the nucleic acid in the method of detection of the nucleic acid using the reaction more nearly electrochemical than fundamental analysis.
[0035] y=0.72x+8.0 .... (1)
Here, the logarithm of the area (cm2) of the field where y fixes a nucleic acid, and x are the logarithms of detection sensitivity (copy/mL).
[0036] Therefore, it is 7x10 to 4 cm2 about the area of the field which fixes a nucleic acid in an electrode from a formula (1) in order to attain detection of the nucleic acid for example, below 105 copy/mL. It is necessary to make it below. Thus, the minimum area required to fix the 1st nucleic acid is computable by setting up the limit of detection of the 2nd nucleic acid from a formula (1). By the way, photo lithography can be used as a means to produce uniformly a minute field which was mentioned above. In order to raise detection sensitivity so that clearly from a formula (1), it is necessary to make still minuter area of the field which fixes the 1st nucleic acid. However, even if it uses photo lithography, it is ten to 8 cm2. It is difficult to produce the following fields with sufficient repeatability, and repeatability may fall depending on the case. Therefore, the area of the field which fixes the 1st nucleic acid is ten to 8 cm2. It is desirable that it is above. If area of the field which fixes the 1st nucleic acid is made small here, since the amount of the nucleic acid (probe) fixed by the electrode will become less, when the reaction time which hybridization takes is short, formation of a specific hybrid decreases, and the absolute magnitude of the signal acquired may also decrease. In this case, the absolute magnitude of a signal is securable by lengthening time performing hybridization.
[0037] the area asked for the area of the field where an electrode fixes the 1st nucleic acid in the detection equipment concerning this application the 3rd invention corresponding to the limit of detection of the 2nd nucleic acid -- even having -- it is possible to take the composition of the electrode which especially limitation will not be carried out if it is, for example, is built over this application the 1st invention Moreover, about the reaction section, an impression means, and a measurement means, the same composition and same technique as the detection equipment concerning this application the 2nd invention mentioned above can be taken.
[0038] Moreover, while the detection equipment concerning this application the 4th invention fixes the 1st electrode which fixed the 1st nucleic acid, and the 2nd nucleic acid It is characterized by providing the 2nd electrode arranged so that the 3rd nucleic acid can form a hybrid to the above 1st and the 2nd nucleic acid of the above, the power supply connected to the above 1st and the 2nd electrode of the above, and a measurement means to measure the signal produced by operation of the aforementioned power supply.
[0039] It arranges so that the 3rd nucleic acid can form a hybrid to the 1st and the 2nd nucleic acid of the above. the 1st and 2nd electrodes which fixed the 1st and 2nd nucleic acids according to the detection equipment in this application the 4th invention -- this -- Since the signal which originates in the 3rd nucleic acid by measuring the signal which impressed voltage and was produced from the power supply connected to the 1st and 2nd electrodes can be acquired certainly, reducing the background While excelling in repeatability and fixed quantity nature, it becomes possible to detect the 3rd nucleic acid by high sensitivity. Moreover, since shortening of time and the improvement in operability which detection takes are attained, it becomes possible to detect the 3rd nucleic acid economically.
[0040] The detection equipment in this application the 4th invention tends to consider that a nucleic acid is a conductor, tends to detect discharge (luminescence) of the current or the photon produced by applying voltage to a nucleic acid, and tends to detect the 3rd nucleic acid. That is,

the 3rd nucleic acid's formation of the 1st and 2nd nucleic acids and hybrids constitutes a circuit through the hybrid formation section. If voltage is impressed here from the power supply connected to the 1st and 2nd electrodes, current will flow through the 3rd nucleic acid. Then, if electric signals, such as current value, electrical conductivity, potential, resistance, electric capacity, an inductance, and an impedance, are detected, it will mean detecting only the signal originating in the 3rd nucleic acid. Moreover, if current flows through the 3rd nucleic acid, from the 1st, the 2nd, and 3rd nucleic acids in which the hybrid was formed, a photon will be emitted and a nucleic acid will emit light. Then, if luminescence intensity is measured, it will mean detecting the signal originating in the 3rd nucleic acid. Therefore, by measuring an electric or optical signal, while excelling in repeatability and fixed quantity nature, it becomes possible to detect the 3rd nucleic acid by high sensitivity.

[0041] In the detection equipment concerning this application the 4th invention, although the 1st and 2nd electrodes are not limited if it has the 1st and 2nd nucleic acids fixed, the electrode by this application the 1st invention and the electrode applied to this application the 2nd invention can be suitably used for them from the purpose which raises repeatability and fixed quantity nature on the occasion of detection of a nucleic acid. Furthermore, the electrode applied to this application the 3rd invention can be suitably used from a viewpoint which prevents generating of the background. The array of the 1st fixed in the 1st and 2nd electrodes and 2nd nucleic acids may show one kind of base sequence respectively, and may consist of two or more kinds of base sequences. Moreover, generally, although the array of the 1st and 2nd nucleic acids is chosen so that it may differ, you may make the same the array of the 1st and 2nd nucleic acids. Although the physical relationship of the 1st and 2nd electrodes will not be limited if it is arranged in the position which can form the 3rd nucleic acid and hybrid, in order to urge formation of the stable hybrid, arranging face to face is desirable. When the 1st and 2nd electrodes have been arranged face to face, the interval of the 1st and 2nd electrodes is determined depending on the length of the 3rd nucleic acid. Namely, since the 3rd nucleic acid forms a hybrid in the both sides of the 1st and 2nd nucleic acids, and the field of an edge (5'edge and 3'edge) In the length of the 1st, the 2nd, and 3rd nucleic acids, the interval L of the A, B and C then the 1st, and 2nd electrodes is set up so that it may become the range of C+2 alpha<=L<A+B+C +2alpha (the length of the surplus by the thiol group by which alpha was introduced into the 1st and 2nd nucleic acids). however, in order for the 3rd nucleic acid to stabilize and combine with the 1st and 2nd nucleic acids The 1st and 2nd nucleic acids and 3rd nucleic acids need to form a hybrid over about 5-30 bases. Possibility of causing formation of an un-unique hybrid if the field which forms a hybrid in the 1st and 2nd nucleic acids exceeds 30 bases highly moreover, from a bird clapper As for the interval L of the 1st and 2nd electrodes, it is desirable to be adjusted in the range of L=A+B+C+2alpha- (the length of ten bases - 60 base). Specifically, the interval L of the 1st and 2nd electrodes is 50nm - 1 micrometer preferably 10nm - 1mm. It is set as a grade. Moreover, in order to arrange the 1st and 2nd electrodes so that the 1st and 2nd nucleic acids and 3rd nucleic acids may form a hybrid, it is good to use photo lithography for the interior of a capillary tube, and to make the 1st and 2nd electrodes. According to such composition, hybridization can be performed inside the capillary tube which has arranged the 1st and 2nd electrodes, and washing of the 1st and the 2nd electrode after hybridization and measurement of the signal which impressed voltage and was produced from the power supply connected to the 1st and 2nd electrodes can also be performed easily. Furthermore, especially limitation will not be carried out if voltage can be impressed to the 1st and 2nd nucleic acids and the 3rd nucleic acid to which hybrid formation was performed as a power supply connected to the above 1st and the 2nd

electrode of the above. Moreover, the same composition and same technique as the detection equipment applied to this application 2nd mentioned above and the 3rd invention as a measurement means to measure the signal produced by operation of a power supply can be taken.

[0042] Furthermore, the sensor concerning this application the 5th invention is characterized by providing the 1st electrode which fixed the 1st nucleic acid, and the 2nd electrode arranged so that the 3rd nucleic acid can form a hybrid to the above 1st and the 2nd nucleic acid of the above while fixing the 2nd nucleic acid.

[0043] According to the sensor in this application the 5th invention, it becomes possible to form easily the hybrid by which the 3rd nucleic acid was stabilized to the 1st and 2nd nucleic acids by having arranged the 1st and 2nd electrodes which fixed the 1st and 2nd nucleic acids so that the 3rd nucleic acid can form a hybrid to the 1st and 2nd nucleic acids.

[0044] In the sensor in this application the 5th invention, although the 1st and 2nd electrodes are not limited if it has the 1st and 2nd nucleic acids fixed, the electrode by this application the 1st invention can be suitably used for them from the purpose which raises repeatability and fixed quantity nature on the occasion of detection of a nucleic acid. Furthermore, the electrode applied to this application the 3rd invention can be suitably used from a viewpoint which prevents generating of the background. Moreover, although the physical relationship of the 1st and 2nd electrodes will not be limited if the 3rd nucleic acid is arranged to the 1st and 2nd nucleic acids in the position which can form a hybrid, it can take the same composition and same technique as the detection equipment concerning this application the 4th invention, for example.

[0045] Next, the adjustment method of the nucleic acid set as the object of detection is explained.

[0046] Generally, on the occasion of adjustment of a nucleic acid, a whole blood, a blood serum, a leucocyte, a cell, an organization, expectoration, urine, sperm, saliva, various food groups, soil, water, seawater of a river, etc. are used as a sample. And you have to extract the nucleic acid of a minute amount efficiently by any case. For example, in inspection of hepatitis C, you have to extract the nucleic acid of the virus of ten numbers from some which are contained in blood serum 1mL. Moreover, since E. coli bacillus O-157 is infected in existence of hundreds of bacteria, you have to extract the nucleic acid originating in few bacteria adhering to food etc. Usually, in order to extract the nucleic acid of a minute amount and to gather an extraction efficiency, the nucleic acid DNA of a dummy, for example, a salmon sperm, the cow thymus gland DNA, etc. are added as a carrier. However, since the base sequence of this DNA is not known completely, it is the same as the nucleic acid set as the object of detection in the invention in this application, or the analogous array may be included. When a carrier nucleic acid has the nucleic acid (probe) and analogous array which were fixed by the conductor and the electrode, not the nucleic acid used as the candidate for detection but the added carrier nucleic acid will react with the nucleic acid (probe) fixed by the conductor and the electrode. Therefore, even if it is the case (negative) where the nucleic acid which serves as a candidate for detection in fact does not exist, the judgment (false positivity) in which the nucleic acid used as the candidate for detection exists accidentally (positivity) will be made. Generating of the above-mentioned false positivity poses a big problem now [when detection of the nucleic acid which exists in low concentration extremely is called for]. Moreover, in case formation of the hybrid between nucleic acids detects a nucleic acid, it poses a problem that a reaction with the nucleic acid which serves as a nucleic acid (probe) fixed by the conductor and the electrode and a candidate for detection by self hybridization of a nucleic acid own [used as the candidate for detection] is checked. Namely, since it is DNA of the double strand which combined the nucleic acid

complementary mutually except for some viruses, even if it makes a single strand dissociate by heat or alkali at once, the nucleic acid used as the candidate for detection will be restored to DNA of not a probe but the original double strand in the case of hybridization. This reaction tends to occur, when there are comparatively many amounts of the nucleic acid used as the candidate for detection, and it becomes the factor which the detection range by the side of high concentration is narrowed, and reduces fixed quantity nature. Then, the nucleic acid which consists of a known base sequence as a carrier nucleic acid in case a nucleic acid is extracted from a sample, For example, the compound gay oligonucleotides, such as polyA and polyT, Oligonucleotides including the compound hetero, such as poly (AT) The oligonucleotide which the adenine (A), the thymine (T), the guanine (G), and the cytosine (C) arranged at random, If nucleic acids currently generally used, such as a decomposition product of a plasmid or a plasmid, are added so that it may become about 0.1 ng/ml-100 ng/ml, and a nucleic acid is extracted, even if it is the nucleic acid of a minute amount, it can extract from a sample efficiently. However, the array of the nucleic acid added in case it extracts a nucleic acid from a sample, in performing high bull die ZESHON using the nucleic acid extracted according to the above-mentioned method must check being [of the nucleic acid (probe) used in the case of detection] an array, and that it is not complementary. Moreover, the carrier nucleic acid to add is not necessarily limited to one kind, and can add two or more kinds of carrier nucleic acids in one sample. Moreover, although especially the molecular weight of a carrier nucleic acid is not limited, either, the range of about 1000 bases of a number base to numbers is desirable. It is also possible for especially the method of extracting a nucleic acid from a sample not to be limited, and to use the kit of marketing, such as solvent extraction, the solid-liquid extraction method using support or QIAamp(s) (product made from QIAGEN), such as the phenol-chloroform method, and SUMAITESU ** (Sumitomo Metal Industries, Ltd. make).

[0047] The extracted nucleic acid can be used not only for the invention in this application but for various kinds of uses. For example, the dot blotting technique using the probe which carried out the indicator with RI or the fluorescent substance, a Southern blot technique, the NO 1 ZAMBU lot method, and the microplate method can be mentioned. Moreover, a probe side can also be used for the method of detecting the type fixed in support. In addition, when detecting the nucleic acid adjusted by this method using the electrode, the sensor, or detection equipment of the invention in this application, the nucleic acid set as the object of detection is a double strand in many cases, and a reaction with the nucleic acid (probe) fixed on the conductor or the electrode by the self hybridization between the nucleic acids set as the object of detection in this case will be checked. However, if the carrier nucleic acid added when extracting a nucleic acid from a sample has a complementary array or an analogous array in a part of nucleic acid set as the object of detection, a hybrid is formed between the nucleic acids set as a carrier nucleic acid and the object of detection in the field of this array, and the self hybridization between the nucleic acids set as the object of detection can be stopped. At this time, if a carrier nucleic acid is made into the length of about ten bases of numbers from a number base in consideration of the reactivity between the nucleic acids set as a carrier nucleic acid and the object of detection, it is effective. In addition, the carrier nucleic acid of being chosen so that formation of the hybrid between the nucleic acids set as the nucleic acid (probe) fixed on the conductor or the electrode and the object of detection may not be barred is natural. Thus, it is complementary to the nucleic acid set as the object of detection in the base sequence of a carrier nucleic acid, and it becomes possible [the nucleic acid fixed on the conductor or the electrode] it not only to raise the efficiency of extraction of the nucleic acid from a sample, but to suppress formation of the

hybrid between the nucleic acids set as the object of detection, and to detect a quantitive nucleic acid, if it is set as the array which does not form a hybrid (probe). If the extraction method of the nucleic acid mentioned above is applied to the detection of a nucleic acid which used the electrode, the optical fiber, the quartz resonator, etc., it is very effective. The principle of detection of the nucleic acid using the electrode, the optical fiber, the quartz resonator, etc. is the nucleic acid set as the object of detection having been fished, and measuring directly or indirectly a subsequent change of state (change to a double strand from a single strand, and change of a weight), and reading it by the nucleic acid (probe) fixed by these. Then, if the nucleic acid set as the object of detection and a carrier nucleic acid with a complementary array (except for the array of the field which forms a hybrid, the nucleic acid, i.e., the probe, fixed by the electrode, the optical fiber, the quartz resonator, etc.) are used, a carrier nucleic acid will hybridize into the unreacted portion (usually with a single strand) of the nucleic acid set as the object of the fished detection, and a double strand will be formed partially. Consequently, the state of the nucleic acid of tops, such as an electrode, an optical fiber, and a quartz resonator, is made to change a lot, and the signal which detection takes is increased. That is, if it is the case where a quartz resonator is used, the variation of the weight on this quartz resonator will become large, and the frequency decrement which is a detecting signal will increase. Moreover, if it is the case where an electrode is used, the electrochemical signal used for measurement by the increase in the double stranded DNA on an electrode will increase. Furthermore, when using the intercalating agent intercalated in the double chain of a nucleic acid, for example, the HOECHST 33258, as an indicator agent, since combining with AT array is known, if this agent sets up the array of a carrier nucleic acid to an AT-rich field, the amount of combination of the HOECHST 33258 can be increased and the signal at the time of measurement will be amplified still more effectively. Moreover, amplification of a signal is further expectable if a part or all uses that by which the indicator is carried out by the indicator agent as a carrier nucleic acid. Although especially the indicator agent to be used is not limited, various kinds of indicator agents mentioned above can be suitably used for it. As mentioned above, the extraction efficiency of the nucleic acid from a sample is raised by extracting a nucleic acid from a sample by the method mentioned above, and when hybridization detects existence of the target nucleic acid using the this extracted sample, while preventing the false positivity by existence of a similar array, self hybridization of a nucleic acid own [target] is suppressed, and it becomes possible to increase the signal which measurement takes.

[0048] Next, in the detection equipment by this application 2nd - the 4th invention, the conditions at the time of performing hybridization are explained.

[0049] Hybridization is usually performed in ionic strength 0.01-5 and the buffer solution of the range of peta5-10. In order to prevent decomposition of carrier nucleic acids, such as the sulfuric-acid dextran and the salmon sperm DNA which are the accelerator of hybridization, and the cow thymus gland DNA, and a nucleic acid in the buffer solution, it is possible to add EDtaualpha, a surfactant, etc. In addition, it is good to add the carrier nucleic acid which expects prudence for addition of a carrier nucleic acid since hybridization may be checked when a carrier nucleic acid is intermingled in case hybridization is performed as mentioned above, and consists of a known base sequence if needed. On the other hand, thermal denaturation of the nucleic acid extracted from the sample is carried out above 90 degrees C, and it mixes in the buffer solution. In addition, after carrying out thermal denaturation of the nucleic acid extracted from the sample above 90 degrees C, it can quench at 0 degree C and can also mix in the buffer solution. Among a reaction, it is also possible to operate stirring or shake and to raise a reaction rate. And

hybridization is performed by the reaction time of about one evening 1 minute or more under 10-90 degrees C. By the way, as mentioned above, the nucleic acid (probe) fixed to the electrode makes the nucleic acid used as the candidate for detection about [15 base -3000 bases] length in many cases, in order to detect certainly, without producing the background. And the base sequence of the nucleic acid (probe) fixed to the electrode is designed so that the nucleic acid used as this nucleic acid (probe) and the candidate for detection can form a hybrid specifically, and a base sequence and a complementary strand characteristic of the nucleic acid used as the candidate for detection may be formed. Although the reaction temperature mentioned above chooses an optimum value by the homology with the nucleic acid set as the base sequence of the nucleic acid (probe) fixed to the electrode, and the object of detection, generally the suitable reaction temperature which faces hybridization is set as temperature lower 20-25 degrees C than the temperature (Tm) to which a nucleic acid denatures (dissolution). Here, Tm is computable from the following formulas.

[0050] The concentration of Tm=81.5+16.6 (log 10[Ci])+0.41 (% G+C)-0.63(% formamide)-(600/n)-1.5 (% mismuch), however a cation with monovalent Ci and n are the numbers of bases of a probe.

[0051] Moreover, the following formulas can be used as the simple calculation method of Tm.

[0052] Tm= 2 degree-C(A+T) %+4 degree-C(G+C) % (when a probe is below 18 mer especially)

When it is set as temperature lower 20-25 degrees C than Tm on the occasion of hybridization, the balance of a reaction inclines toward the about 2 chain formation side. Therefore, formation of a specific hybrid increases with time by lengthening reaction time. Moreover, it is also possible to promote formation of a specific hybrid electrochemically (JP,5-199898,A). If plus potential is impressed to the electrode which fixed the nucleic acid (probe) in the case of hybridization, since DNA and RNA in reaction mixture are charged in negative, they can draw DNA and RNA near near the electrode. Therefore, the concentration of DNA or RNA becomes high [near the nucleic acid (probe) fixed in the electrode]. The detection reaction of the nucleic acid in the inside of the liquid phase is analyzed by Marmur and others in 1960 (Proc.Natl.Acad.Sci.USA, 46, 456, 1960), and it is shown to the concentration of a nucleic acid that it is a secondary reaction. Therefore, a reaction rate will become quick if the concentration of a nucleic acid becomes high near the electrode. That is, hybridization can be promoted electrochemically. In addition, in the detection equipment concerning this application the 4th invention, after impressing plus potential to the 1st electrode and performing hybridization, it is good to impress plus potential to the 2nd electrode and to be made to perform hybridization. In addition, before measuring by the measurement means after performing hybridization in order to reduce the background at the time of measurement, as for an electrode, it is desirable to wash with the buffer solution (for example, hybridization solution), the pure water which sterilized.

[0053]

[Embodiments of the Invention] The composition of the composition of the electrode of the invention in this application and a sensor, the creating method, and detection equipment is explained one by one.

[0054] (Composition of an electrode) Drawing 1 and drawing 2 are cross sections (drawing 1 (b) and drawing 2 (b)) when the plan (drawing 1 (a) and drawing 2 (a)) and A-B line which show the composition of the electrode concerning one operation form of the invention in this application cut. In drawing 1 , the conductor 11 is formed on the substrate 12 which makes the foundation of an electrode. And after making an insulator 14 form in a part of conductor 11 and

substrate 12, the opening 13 which consists of an exposed conductor which carried out opening by photo lithography is formed. Moreover, in drawing 2 , the glue line 15 is first formed on the substrate 12 which makes the foundation of an electrode. And after making a conductor 11 form on a glue line 15, the opening 13 which consists of an exposed conductor which carried out opening by photo lithography is made to form. furthermore, operative condition with this invention another [drawing 3 and drawing 4] -- it is the electrode built like and is the electrode which can be used for inspection simultaneously conducted using one kind of probe to the inspection or the number sample using one or more kinds of probes at once Although drawing 3 and the fundamental structure of the electrode of drawing 4 are the same as an electrode given in drawing 1 or drawing 2 , they have two or more openings 31. Although it is the composition of detecting a nucleic acid as two or more openings 31 of the diameter of the same are formed in the electrode shown in drawing 3 and the same sample corresponds to one opening 31 The electrode shown in drawing 4 is the composition of detecting a nucleic acid from the purpose which raises fixed quantity nature as opening 31 and opening 32 which consist of two paths are made into one group and the same sample corresponds to two openings 31 and opening 32. In addition, naturally in the electrode in drawing 4 , the amounts of the nucleic acid (probe) fixed by opening 31 and opening 32 differ. Furthermore, drawing 5 is an electrode concerning still more nearly another embodiment of the invention in this application, and is the electrode of reference electrode one apparatus which has the reference electrode 52 which is not fixing the probe to opening 51. Although the fundamental structure of the electrode shown in drawing 5 is the same as an electrode given in drawing 1 or drawing 2 , a nucleic acid (probe) is not fixed to a reference electrode 52, but it has two or more openings 51 as well as the electrode shown in drawing 3 . In addition, the surface area of opening [in / drawing 1 - drawing 5 / as mentioned above] is 7x10 to 4 cm2, when attaining detection of the surface area set up from the formula (1) based on limit of detection, for example, the nucleic acid below 105 copy/mL. Being adjusted so that it may become cannot be overemphasized. Moreover, drawing 6 is drawing having shown 1 operation form of the detection equipment which used the above-mentioned electrode. In drawing 6 , the electrode which fixed the nucleic acid 62 from which 61 becomes a probe, and 63 are reaction vessels, and they are connected so that a power supply 64 may form a circuit through an electrode 61 and a reaction vessel 63. Moreover, inside the circuit, an ammeter 65, a voltmeter 66, and variable resistance 67 are arranged, and after performing hybridization within a reaction vessel 63, it is constituted so that the current which impresses voltage and flows in a circuit may be measured. In addition, it is possible to be able to replace easily the solution held inside a reaction vessel 63, and to replace the interior of a reaction vessel 63 with a fresh hybridization solution etc. at the time of measurement. According to the detection equipment in drawing 6 , the buffer solution (hybridization solution) and the sample which were mentioned above inside the reaction vessel 63 are made to hold, and hybridization of a sample and the nucleic acid 62 is carried out. As for the temperature and time of hybridization, at this time, it is desirable to be set up as mentioned above. Subsequently, preferably, a reaction vessel 63 and an electrode 61 are washed, a solution (for example, hybridization solution) fresh to the interior of a reaction vessel 63 is filled, a power supply 64 is operated, and current value or a voltage value is measured using an ammeter 65 or a voltmeter 66. And the amount (concentration) of the nucleic acid made applicable [which is contained in a sample] to detection is computed from a measurement result. In addition, as mentioned above, it is possible to raise detection sensitivity more using various kinds of indicator agents.

[0055] Moreover, drawing 7 and drawing 8 are drawings of the detection equipment completely

automated using the above-mentioned electrode showing composition [like] 1 operative condition.

[0056] The electrode holder which possessed the electrode of the above [detection equipment] in drawing 7 and drawing 8 , The reaction section possessing the temperature adjustable reaction vessel for making the above-mentioned electrode and a sample react, The first washing section possessing the temperature adjustable washing tub for washing an electrode after a reaction (hybridization), The intercalating-agent reaction section possessing the temperature adjustable intercalating-agent solution tub for making the washed electrode after a reaction (hybridization) react with an intercalating agent, The second washing section possessing the temperature adjustable washing tub for washing an electrode after the reaction by the intercalating agent, The electrochemical test section for performing electrochemistry measurement of the washed electrode after the reaction by the intercalating agent, The analysis unit for analyzing the data obtained by the aforementioned electrochemical measurement, It has move equipment which adjoined and carried the operation unit for controlling a series of above-mentioned reactions (hybridization), washing, the reaction by the intercalating agent, washing, and electrochemical measurement, the aforementioned reaction section, the first washing section, the intercalating-agent reaction section, the second washing section, and the electrochemical test section. In addition, the carrying-in mouth which carries in the sample further for detection (sample) to the above-mentioned detection equipment, The sample adjustment unit for extracting the nucleic acid for making it react with an electrode from a sample, The conveyance unit which moves the sample between this carrying-in mouth and this sample preparation unit and between this sample preparation unit and the aforementioned reaction section, It is desirable to have the waste storage unit of a mind [of keeping the waste fluid produced in the aforementioned reaction (hybridization), washing, and intercalating-agent reaction time] sake.

[0057] The detection equipment 70 shown in drawing 7 possesses the move equipment 77 which carried the electrode fixed electrode holder 71, the reaction vessel 72, the 1st washing tub 73, the intercalating-agent reaction vessel 74, the 2nd washing tub 75, the electrochemistry measurement tub 76 and the aforementioned reaction vessel 72, the washing tub 73, the intercalating-agent reaction vessel 74, the washing tub 75, and the electrochemistry measurement tub 76 for holding the electrode explained above, the analysis unit 78, and the operation unit 79. Movement of the electrode between these tubs is attained by moving a reaction vessel etc. using move equipment 77. The solution of the nucleic acid beforehand extracted from the sample is put into the reaction vessel 72, and the electrode of the invention in this application fixed to the electrode fixed electrode holder 71 is dipped in a reaction vessel 72. Subsequently, the denaturation process of a nucleic acid, the process which performs hybridization of the nucleic acid which denaturalized, and the electrode which fixed the probe, a washing process, an intercalating-agent reaction process, a washing process, and an electrochemistry process are performed one by one in a reaction vessel 72. At a denaturation process, 90 degrees C or more 98 degrees C or less, after heating preferably the nucleic acid extracted from the sample at 95 degrees C or more 98 degrees C or less, it is quenched. At the process which performs the following hybridization, it reacts by controlling **0.5 degrees C of temperature by level of **0.1 degrees C preferably between 30 degrees C and 80 degrees C. It washes in the following first washing tub 73, carrying out the temperature control of the electrode between 25 degrees C and 80 degrees C. According to this process, the nucleic acid combined with the electrode in un-unique can be removed. Next, a washed electrode is controlled by temperature between 25 degrees C and 80 degrees C, and the reaction of an intercalating agent and the double strand nucleic acid formed on the electrode is

made to perform by the intercalating-agent solution tub 74. It washes in the following second washing tub 75, carrying out the temperature control of the electrode between 25 degrees C and 80 degrees C. Furthermore, electric measurement is performed by the electrochemistry measurement tub 76. After a measurement end, the calibration curve currently beforehand recorded on the analysis unit 78 is made reference, and the concentration of the nucleic acids for [in a sample] detection (purpose gene etc.) is outputted. The above operation is performed through the operation unit 79 electrically connected with each tub and unit. Moreover, in addition to the detection equipment shown in drawing 7 , the detection equipment 80 shown in drawing 8 is equipped with the sample conveyance unit 82 which connects between the sample carrying-in mouth 81, the sample preparation unit 83, and these sample carrying-in mouths 81 and these sample preparation units 83 and between this sample preparation unit 83 and the above-mentioned detection equipment 70, and the waste storage unit 87. In this mode, after paying a sample to the sample carrying-in mouth 81, it is carried to the sample preparation unit 83 through the sample conveyance unit 82, and extraction of a nucleic acid is performed in this sample preparation unit 83. The extracted nucleic acid is carried by above detection equipment 70 through this sample conveyance YUNITSU ** 82, and as mentioned above, detection of a nucleic acid is performed. In addition, the waste produced in the washing process etc. to the waste storage unit 85 is saved temporarily.

[0058] Furthermore, drawing 9 and drawing 10 are drawings having shown 1 operation gestalt of the sensor concerning the invention in this application. In drawing 9 and drawing 10 , a nucleic acid (probe) 95, the nucleic acid 96, the nucleic acid 107, the nucleic acid 108, and the nucleic acid 109 are respectively fixed on the conductor 93 arranged on a substrate 91, a substrate 92, a substrate 101, a substrate 102, and a substrate 103, the conductor 94, the conductor 104, the conductor 105, and the conductor 106. Especially the sensor by drawing 10 can be used for the inspection using two or more kinds of probes at once. Drawing 9 and the fundamental structure of the electrode in the sensor of drawing 10 can use the electrode of a publication for drawing 1 - drawing 4 suitably, although especially limitation is not carried out. And as shown in drawing 9 (b) and drawing 10 (b), the nucleic acid 110 used as the candidate for detection forms a hybrid so that it may straddle by the both ends of a nucleic acid 110 between a nucleic acid 95, a nucleic acid 96 and a nucleic acid 107, a nucleic acid 108 or a nucleic acid 107, and a nucleic acid 109. Moreover, when a hybrid is formed so that the nucleic acid 110 used as the candidate for detection may straddle between a nucleic acid 95, a nucleic acid 96 and a nucleic acid 107, a nucleic acid 108 or a nucleic acid 107, and a nucleic acid 109, as shown in drawing 11 , the nucleic acid 111 which carried out the indicator by the indicator agent from the purpose which raises detection sensitivity can also be combined with the nucleic acid 110 used as the candidate for detection. Moreover, in drawing 9 - drawing 11 , when the surface area of the field which fixed the nucleic acid on an electrode is adjusted so that it may become the surface area set up from the formula (1) based on limit of detection, it is more desirable. Furthermore, in order to hold the gap of a substrate 91 and a substrate 92 certainly so that it may become fixed as shown in drawing 11 , it is desirable to form a spacer 112 between a substrate 91 and a substrate 92. In case this spacer arranges a conductor on an electrode, it can use for and form photo lithography.

[0059] Moreover, drawing 12 is drawing having shown the electric composition of the detection equipment which used the above-mentioned sensor. In drawing 12 , to the electrode 121 and electrode 122 which fixed the nucleic acid 123 and nucleic acid 124 used as a probe, it connects so that a power supply 125 may form a circuit through an electrode 121 and an electrode 122. Moreover, inside the circuit, an ammeter 126, a voltmeter 127, and variable resistance 128 are

arranged, and after performing hybridization through an electrode 121 and an electrode 122, it is constituted so that the current value which impresses voltage and flows in a circuit may be measured. Furthermore, drawing 13 is drawing having shown one embodiment of the detection equipment of the invention in this application which used the above-mentioned sensor. In drawing 13 , the electrode 131 and the electrode 132 have the composition that it is made inside the capillary tube 133 which consists of quartz glass etc., and hybridization is performed inside a capillary tube 133. It connects with the electrode 131 and the electrode 132 so that a power supply 134 may form a circuit through an electrode 131 and an electrode 132, and inside the circuit, an ammeter 135, a voltmeter 136, and variable resistance 137 are arranged. In addition, in the detection equipment in drawing 13 , the solution of a sample has composition which circulates the interior of a capillary tube 133 in the fixed direction. Then, as shown in drawing 14 , adjustment of the timing of pouring of the sample from operation of the liquid-sending pump 141 and the pouring section 142 of a sample is enabled. After circulating a sample inside a capillary tube 133, stop circulation of the solution of a sample and hybridization is performed. After hybridization is completed, by constituting the interior of a capillary tube 133 so that a fresh solution may circulate, the electrode after hybridization and the interior of a capillary tube 133 can be washed easily. Therefore, a nucleic acid with more high fixed quantity nature is detectable with a measuring device 143. In addition, the equipment which detects the photon produced from the equipment or the nucleic acid which measures an electrochemical signal is sufficient as a measuring device 143, and the equipment which combined these is sufficient as it. Moreover, in order to control the temperature at the time of hybridization correctly, it is also possible to arrange heating meanses, such as a heater controlled by the thermostat etc. around a capillary tube 133. Drawing 15 is drawing having shown one embodiment of the detection equipment which applied the composition shown in above-mentioned drawing 13 and drawing 14 . Detection equipment 150 the pouring section 153 which holds and pours in the solution tank 152 and sample holding the fixed electrode holder 151 for holding the sensor shown in above-mentioned drawing 9 and drawing 10 , and the buffer solution (hybridization solution) The fixed electrode holder 151 is minded. The solution after hybridization The output terminal 157 for outputting the result measured from the liquid-sending line 155 linked to the waste fluid tank 154 to save, the measurement unit 156 which measures the signal from the fixed electrode holder 151 holding the sensor, and the measurement unit 156 outside is provided. A sample and the buffer solution (hybridization solution) move through the liquid-sending line 155 with the driving force of the liquid-sending pump 158. Although the buffer solution (hybridization solution) is sent toward the fixed electrode holder 151 from the solution tank 152 through the liquid-sending line 155, the solution of the nucleic acid beforehand extracted from the sample is mixed from the pouring section 153 in the process. If the buffer solution (hybridization solution) which mixed the nucleic acid extracted from the sample reaches the sensor with which the fixed electrode holder 151 was equipped, it will be stopped and liquid sending through the liquid-sending line 155 will perform the process which performs hybridization between the denaturation process of a nucleic acid, and the nucleic acid which this denatured and the sensor which has the electrode which fixed the probe one by one in the fixed electrode holder 151. before [in addition,], as for the denaturation process of a nucleic acid, a nucleic acid reaches the fixed electrode holder 151 -- you may carry out -- for example, denaturation -- you may make it pour in a nucleic acid from an inlet 153 the bottom After hybridization is completed in the fixed electrode holder 151, the buffer solution (hybridization solution) is again sent toward the fixed electrode holder 151 through the liquid-sending line 155, and while washing the sensor with which the fixed electrode

holder 151 was equipped, electrochemical or optical measurement by the measurement unit 156 is performed. In addition, before washing the sensor with which the fixed electrode holder 151 was equipped, you may carry out the reaction process by the intercalating agent. As mentioned above, after heating preferably the nucleic acid extracted from the sample at 95 degrees C or more 98 degrees C or less, at a denaturation process, it is quenched 90 degrees C or more 98 degrees C or less. At the process which performs the following hybridization, it reacts by controlling **0.5 degrees C of temperature by level of **0.1 degrees C preferably between 30 degrees C and 80 degrees C. Moreover, it washes at the time of washing of the fixed electrode holder 151, carrying out the temperature control of the sensor between 25 degrees C and 80 degrees C. Therefore, the heating means in which a temperature control is possible is installed in the interior of the fixed electrode holder 151. Furthermore, the concentration of the nucleic acids for [in a sample] detection (purpose gene etc.) is computed by the electric or optical measurement result by the measurement unit 156 being outputted outside from an output terminal 157, and making reference the calibration curve currently searched for beforehand.

[0060] (Conclusion of an operation gestalt) It is as follows when the composition of the electrode shown in the gestalt of operation, a sensor, and detection equipment, an operation, and an effect are summarized.

[0061] (1) The electrode (drawing 1 - drawing 5) shown in the operation gestalt A substrate, the conductor arranged on this substrate, and the insulator which was wearing some front faces of this conductor and was formed, Opening formed in this insulator so that the aforementioned conductor might be exposed with photo lithography, The nucleic acid (probe) fixed to the conductor exposed from this opening is provided, and an electrical signal, an optical signal, etc. which are produced when a nucleic acid with the predetermined array specifically combined with this nucleic-acid probe combines with a probe can be detected now. The amount of the nucleic acid (probe) by which the above-mentioned electrode is fixed on the conductor which it is correctly controlled in accordance with the limit of detection of the nucleic acid from which the area of opening serves as a candidate for detection, consequently was exposed from opening since opening was exposed by photo lithography is controlled correctly. Furthermore, the electrode (drawing 2) shown in the operation gestalt has the glue line between the substrate and the conductor. In this electrode, although excelled as the quality of the material for electrodes, an adhesive property with a substrate can create the electrode which was more stable and was excellent using a bad conductor. Moreover, the above-mentioned electrode shown in the operation gestalt is what used the resin for the insulator, and has become a thing using the photopolymer or the photoresist preferably also in the resin. It is the thing and intermediary **** which used any of the photoresist for optical exposure, the photoresist for far-ultraviolet, the photoresist for X-rays, and the photoresist for electron rays they were still more preferably. In the above-mentioned electrode, a resist can be used as an insulator as it is after patterning by lithography, and it is possible to lessen the process which is in charge of electrode creation. Moreover, the sensor (drawing 9 - drawing 11) shown in the operation gestalt has two electrodes by which the nucleic acid was fixed respectively, and since the nucleic acid which serves as a candidate for detection through the nucleic acid fixed by this electrode forms a hybrid, fixed quantity nature can detect a nucleic acid to high sensitivity highly by electric or optical measurement. The electrode shown in the operation gestalt is what made the orientation index of (111) of an electrode three or less [1 or more], and what used gold also in the electrode is desirable. In the above-mentioned electrode, since a sulfur atom and an interaction have the strong field (111) of an electrode, it is possible to fix the thiol-ized nucleic-acid probe in an

electrode efficiently.
[0062] (2) Since the detection equipment (drawing 6 , drawing 13 - drawing 15) shown in the operation gestalt has taken the composition with which detection of a nucleic acid is carried out using the electrode or sensor mentioned above, fixed quantity nature can detect highly the nucleic acid set as the object of detection to high sensitivity by electric or optical measurement. Moreover, the detection equipment (drawing 7) shown in the operation gestalt The reaction section which is detection equipment possessing the electrode for detecting the nucleic acid which has a predetermined base sequence, and possessed the temperature adjustable reaction vessel for making an electrode and a sample react, The first washing section possessing the temperature adjustable washing tub for washing an electrode after a reaction (hybridization), The intercalating-agent reaction section possessing the temperature adjustable intercalating-agent solution tub for making the washed electrode after a reaction react with an intercalating agent, The second washing section possessing the temperature adjustable washing tub for washing an electrode after a reaction with an intercalating agent, The electrochemical test section for performing electrochemistry measurement of the washed electrode after a reaction with an intercalating agent, The analysis unit for analyzing the data obtained by electrical-and-electric-equipment-ized character-measurement, The operation unit for controlling a series of aforementioned reactions, washing, an intercalating-agent reaction, washing, and electrochemical measurement, By being a thing possessing the move equipment which adjoined and carried the aforementioned gene reaction section, the first washing section, the intercalating-agent reaction section, the second washing section, and the electrical-and-electric-equipment-ized character-test section, and using the move equipment of further the above Reaction on this electrode, washing of this electrode, and electrochemical measurement of this electrode were performed one by one, and the nucleic acid is detected electrically and automatically. Since the unit for the reaction vessel etc. being adjoined and carried on move equipment, and operating and analyzing each reaction etc. is connected so that a series of reactions using the electrode in connection with the detection of nucleic acids (gene etc.) which has a specific array, washing, etc. may be performed one by one, the above-mentioned detection equipment can detect electrically and automatically the nucleic acid which has a predetermined base sequence. Moreover, in addition to above equipment, the automatic gene detection equipment (drawing 8) shown in the gestalt of operation is the thing equipped with the sample conveyance unit which connects between a sample carrying-in mouth, a sample preparation unit, and these sample carrying-in mouths and these sample preparation units and between this sample preparation unit and the above-mentioned detection equipment, and the waste storage unit. Since this detection equipment is equipped with the sample adjustment unit which extracts a nucleic acid from a sample, the sample conveyance unit in connection with transportation between [sample carrying in to] gene detection reaction vessels, and the waste storage unit, it can analyze the nucleic acid which originates altogether from extraction of the nucleic acid from a sample until it results in the detection reaction and analysis of a nucleic acid in two or more samples in succession automatically.
[0063]
[Example]
(Example 1) In this example, the electrode corresponding to the form of operation shown in drawing 2 was manufactured.
[0064] 7. After the sulfuric acid and the hydrogen-peroxide solution washed the Pyrex (registered trademark) glass substrate 12 of 62 cm (3 inches), by the electron beam method,

vacuum evaporationo was carried out so that it might become the thickness of 500A about titanium by the conventional method first, and subsequently gold was similarly deposited so that it might become the thickness of 5000A. Next, lithography of a vacuum evaporationo metal was performed using the photoresist AZ4620 for optical exposure, and the conductor 11 was formed. Subsequently, the same photoresist was applied, exposure development was carried out, and the electrode with the opening 13 (area : 10 -4 cm2) to which a conductor 11 is exposed was formed.
[0065] Subsequently, the nucleic acid (probe) for detection of beta type hepatitis virus (etaBV) was fixed in this electrode (1012 copy/cm2). Moreover, the golden wire was ****(ed) by the resin for comparison, and the electrode which fixed the same nucleic acid (probe) as the electrode in this example (1012 copy/cm2) was used similarly. Fixation of a probe and detection of a nucleic acid were carried out by the method shown in the form of operation. Although dispersion was 10 - 20% in coefficient of variation as a result of the detection experiment in the case of the golden wire type electrode, in the electrode in this example, coefficient of variation was 5% or less.
[0066] (Example 2) In this example, the electrode corresponding to the form of operation shown in drawing 2 was created.
[0067] 7. After the sulfuric acid and the hydrogen-peroxide solution washed HoyaNA-40 substrate 12 of 62 cm (3 inches), by sputtering, membranes were formed so that it might become the thickness of 500A about chromium by the conventional method first, next gold was similarly formed so that it might become the thickness of 5000A. Subsequently, lithography was performed using the photoresist AZ4620 for optical exposure, and the glue line 15 and the conductor 11 were formed simultaneously. Subsequently, the same photoresist was applied, exposure development was carried out, and the electrode with the opening 13 (area : 10 -4 cm2) to which a conductor 11 is exposed was formed.
[0068] Subsequently, the nucleic acid for detection of a tubercule bacillus (probe) was fixed in this electrode (1012 copy/cm2). Fixation of a probe was carried out by the method shown in the form of operation. Moreover, the golden wire was ****(ed) by the resin for comparison, and the electrode which fixed the same nucleic acid (probe) as the electrode in this example (1012 copy/cm2) was used similarly.
[0069] First, from the carrier, expectoration was extracted and the nucleic acid was extracted using the phenol-chloroform method. It dissolved in the solution of the sodium chloride of 150mM, and the sodium citrate (pH 7.0) of 15mM, and the extracted nucleic acid was used for hybridization. After hybridization, electrochemical measurement was performed, after washing the electrode and flooding with the solution of the intercalating-agent HOECHST 33258. Although dispersion was 10 - 20% in coefficient of variation as a result of the detection experiment in the case of the golden wire type electrode, in the electrode in this example, coefficient of variation was 5% or less.
[0070] (Example 3) In this example, the electrode corresponding to the gestalt of operation shown in drawing 2 was created.
[0071] 7. On SiO2 of 62 cm (3 inches), and (111) the substrate 12 of SiO2 (100), by sputtering, membranes were formed so that it might become the thickness of 500A about titanium by the conventional method first, and subsequently, similarly, gold was formed so that it might become the thickness of 5000A. And as a result of measuring the orientation index of the gold (111) on each substrate, on SiO2 (111), it was about 1 on about 2 and SiO2 (100). Next, lithography was performed using the photoresist AZ4620 for optical exposure, and the glue line 15 and the conductor 11 were formed simultaneously. Subsequently, the same photoresist was applied,

exposure development was carried out, and the electrode with the opening 13 (area : 10 -4 cm2) to which a conductor 11 is exposed was formed.
[0072] Subsequently, the nucleic acid (probe) for detecting RNA of hepatitis C virus (etavalve flow coefficient) to this electrode was fixed (1012 copy/cm2). Fixation of a nucleic-acid probe was carried out by the method shown in the gestalt of operation.
[0073] First, from the carrier, the blood serum was extracted and RNA was extracted using the phenol process. It dissolved in the solution of the sodium chloride of 150mM, and the sodium citrate (peta7.0) of 15mM, and the extracted nucleic acid (RNA) was used for hybridization. After hybridization, electrochemical measurement was performed, after washing the electrode and flooding with the solution of the intercalating-agent HOECHST 33258. In the electrode equipped with the SiO2 substrate (100) to the coefficient of variation of the electrode equipped with SiO2 (111) substrate having been 4% as a result of the detection experiment, coefficient of variation was 8%. As mentioned above, when a probe is fixed on a conductor, it turns out that the way which used the high conductor of a stacking tendency is excellent in repeatability.
[0074] (Example 4) In this example, the electrode corresponding to the gestalt of operation shown in drawing 2 was created.
[0075] 7. After the sulfuric acid and the hydrogen-peroxide solution washed the alumina substrate 12 of 62 cm (3 inches), by sputtering, membranes were formed so that it might become the thickness of 500A about titanium by the conventional method first, next gold was similarly formed so that it might become the thickness of 5000A. Subsequently, lithography was performed using the photoresist AZ4620 for optical exposure, and the glue line 15 and the conductor 11 were formed simultaneously. Subsequently, the same photoresist was applied, exposure development was carried out, and the electrode with the opening 13 (area : 10 -4 cm2) to which a conductor 11 is exposed was formed.
[0076] Subsequently, SK38 probe combined with the env gene of human immunodeficiency virus (HIV) was fixed in this electrode (1012 copy/cm2). Fixation of a probe was carried out by the method shown in the gestalt of operation. Moreover, the golden wire was ****(ed) by the resin for comparison, and the electrode which fixed the same nucleic acid (probe) as the electrode in this example (1012 copy/cm2) was used similarly.
[0077] First, from the carrier, the blood serum was extracted and RNA of HIV was extracted using the phenol process. It dissolved in the solution of the sodium chloride of 150mM, and the sodium citrate (peta7.0) of 15mM, and the extracted nucleic acid (RNA) was used for hybridization. After hybridization, electrochemical measurement was performed, after washing the electrode and flooding with the solution of the intercalating-agent HOECHST 33258. Although dispersion was 10 - 20% in coefficient of variation as a result of the detection experiment in the case of the golden wire type electrode, in the electrode in this example, coefficient of variation was 5% or less.
[0078] (Example 5) In this example, the electrode corresponding to the gestalt of operation shown in drawing 3 was created.
[0079] 7. After the sulfuric acid and the hydrogen-peroxide solution washed the Pyrex (registered trademark) glass substrate 34 of 62 cm (3 inches), by sputtering, membranes were formed so that it might become the thickness of 500A about titanium by the conventional method first, and subsequently gold was similarly formed so that it might become the thickness of 5000A. Next, lithography was performed using the photoresist alphaZ4620 for optical exposure, and the pattern of a glue line, a conductor, and lead wire 33 was formed. Subsequently, the same photoresist was applied, exposure development was carried out, and the electrode equipped with

the resist pattern (insulator) with the opening 31 (area : 10 -4 cm2) to which a conductor is exposed was formed. With this lithography, four openings 31 were created on one electrode.
[0080] subsequently, it is the subtype of hepatitis B virus -- adr, adw, and ayr -- and -- The nucleic acid (probe) of 20 bases complementary only to each of ayw was fixed in every one opening 31 on an electrode (1012 copy/cm2). (1 to 1) Fixation of a probe was carried out by the method shown in the gestalt of operation.
[0081] The nucleic acid which originates in hepatitis B virus from the blood serum of a carrier was extracted like the above-mentioned example. After amplifying the extracted nucleic acid by rhoCR so that the field which can form the above-mentioned probe and a hybrid may be included, hybridization was performed like the above. With the blood serum extracted from this carrier, it is a subtype as a result of a detection experiment. The signal was acquired only in opening which fixed the complementary probe in adr. Therefore, the genotype of hepatitis B virus was able to be easily determined by using one electrode.
[0082] (Example 6) In this example, the electrode corresponding to the gestalt of operation shown in drawing 5 was created.
[0083] 7. After the sulfuric acid and the hydrogen-peroxide solution washed the Pyrex (registered trademark) glass substrate 54 of 62 cm (3 inches), by the electron beam method, vacuum evaporationo was carried out so that it might become the thickness of 500A about titanium by the conventional method first, and subsequently gold was similarly deposited so that it might become the thickness of 5000A. Next, lithography was performed using the photoresist AZ4620 for optical exposure, and the pattern of a glue line, a conductor, and lead wire 55 was formed. Furthermore, by CVD, the silicon nitride film was formed so that it might become the thickness of 2000A, and the insulator 53 was made to form. Subsequently, the same photoresist was applied, exposure development was carried out, and the electrode equipped with the resist pattern (insulator) with the opening 41 (area : 10 -4 cm2) to which a conductor is exposed was formed. With this lithography, four openings 51 and one reference electrode 52 were created on one electrode.
[0084] Subsequently, the nucleic acid (probe) for detection of beta type hepatitis virus (etaBV) was fixed to each of the conductor exposed from four openings 51 of this electrode (1012 copy/cm2). Fixation of a probe and detection of a nucleic acid were carried out by the method shown in the gestalt of operation. As a result of the detection experiment, at once, detection of four samples was possible and coefficient of variation was 5% or less in all.
[0085] (Example 7) In this example, the electrode corresponding to the gestalt of operation shown in drawing 3 was created.
[0086] 7. After a sulfuric acid and a hydrogen-peroxide solution wash the Pyrex (registered trademark) glass substrate 34 of 62 cm (3 inches), form silver so that it may become the thickness of 5000A, next it is SiO2. Form membranes, an insulator 32 is made to form so that it may become the thickness of 5000A, the photoresist AZP4620 for optical exposure is used, and it is SiO2. Lithography was performed. With this lithography, four openings 31 were created on one electrode. And finally, AZ remover was used and the resist was removed.
[0087] The detection experiment was conducted like the example 5 using this electrode. With the blood serum extracted from this carrier, it is a subtype as a result of a detection experiment. The signal was acquired only in opening which fixed the complementary probe in adr. Therefore, the genotype of hepatitis B virus was able to be easily determined by using one electrode.
[0088] (Example 8) Detection of HCV was tried from the inside of a blood serum using the detection equipment 80 of a publication in the gestalt of operation. 5'-

CCTGTGAGGAalphaCTACTACTGTC-3' complementary to the non-translating field of HCV was fixed to the electrode as a probe. Fixation of a probe was carried out by the method shown in the gestalt of operation. The temperature of hybridization was set as 53 degrees C, and washing, insertion of the intercalating agent to the field in which the hybrid was formed, and the analysis process were controlled by 37 degrees C, and were performed. Consequently, after setting a sample in detection equipment 80, the fixed quantity of the concentration of the nucleic acid which originates in etavalve flow coefficient in 30 minutes per hour [about] was able to be carried out.

[0089] (Example 9) In this example, the electrode corresponding to the gestalt of operation shown in drawing 2 was manufactured.

[0090] 7. After the sulfuric acid and the hydrogen-peroxide solution washed the Pyrex-glass substrate 12 of 62 cm (3 inches), by the electron beam method, vacuum evaporationo was carried out so that it might become the thickness of 500A about titanium by the conventional method first, and subsequently gold was similarly deposited so that it might become the thickness of 5000A. Next, lithography was performed using the photoresist AZ4620 for optical exposure, and the diameter of opening 13 produced three kinds of electrodes, 1mm, 0.1mm, and 0.05mm.

[0091] Subsequently, it fixed in this electrode by the method of showing the nucleic acid (probe) for detection of beta type hepatitis virus (etaBV) in the gestalt of operation (they are 1012 copy/cm2, 1011 copy/cm2, and 1010 copy/cm2 to order). Fixation of a probe and detection of a nucleic acid were carried out by the method shown in the gestalt of operation.

[0092] First, the blood serum was extracted from the carrier and the nucleic acid which originates in etaBV using the phenol-chloroform method was extracted. It dissolved in the solution of the sodium chloride of 150mM, and the sodium citrate (pH 7.0) of 15mM, and the extracted nucleic acid was used for hybridization. After hybridization, after washing the electrode and flooding with the solution of the intercalating-agent HOECHST 33258, the electrochemical signal originating in the HOECHST 33258 was measured. In addition, in the case of hybridization, the electrode was made to produce the potential of plus 0.5V, and the reaction of hybridization was promoted. And when detection sensitivity was measured about three kinds of electrodes, a result shown in Table 1 was brought, by the electrode with a small area of opening, sensitivity is high and the bird clapper was found.

[0093]

[Table 1]

開口部の直径（mm）	核酸を固定化する領域の面積（cm^2）	検出限界（copy/mL）
1	8×10^{-3}	10^6
0.1	8×10^{-5}	10^5
0.05	2×10^{-5}	5×10^{-4}

(Example 10) In this example, the electrode corresponding to the form of operation shown in drawing 2 was created.
[0094] 7. After the sulfuric acid and the hydrogen-peroxide solution washed HoyaNA-40 substrate 12 of 62 cm (3 inches), by sputtering, membranes were formed so that it might become the thickness of 500A about chromium by the conventional method first, next gold was similarly formed so that it might become the thickness of 5000A. Subsequently, lithography was performed using the photoresist AZ4620 for optical exposure, and the glue line 15 and the conductor 11 were formed simultaneously. Subsequently, the same photoresist was applied, exposure development was carried out, and the electrode with the opening 13 to which a conductor 11 is exposed was formed. Under the present circumstances, circular (the diameter of 1.6mm, a surrounding length of 0.5cm, and area 0.02cm2) and two kinds of electrodes made into the square (1.5x1.5mm, a surrounding length of 0.6cm, and area 0.0225cm2) were produced for the configuration of opening 13.
[0095] Subsequently, the nucleic acid (probe) for detection of beta type hepatitis virus (etaBV) was fixed in these electrodes (1012 copy/cm2 and 1012 copy/cm2). Fixation of a probe and detection of a nucleic acid were carried out by the method shown in the gestalt of operation. And detection sensitivity was measured about each electrode. As shown in Table 2 as a result of a detection experiment, although it was almost the same, when the electrode equipped with circular opening is used, as for detection sensitivity, it turns out by each electrode that dispersion decreases.
[0096]
[Table 2]

開口部の形状	検出限界（copy/mL）	測定結果のばらつき：CV（%）
円形	10^7	5
正方形	10^7	8

(Example 11) In this example, the electrode corresponding to the gestalt of operation shown in drawing 2 was created.
[0097] 7. After the sulfuric acid and the hydrogen-peroxide solution washed HoyaNA-40 substrate 12 of 62 cm (3 inches), by sputtering, membranes were formed so that it might become the thickness of 500A about titanium by the conventional method first, next gold was similarly formed so that it might become the thickness of 5000A. Subsequently, lithography was performed using the photoresist AZ4620 for optical exposure, and the glue line 15 and the conductor 11 were formed simultaneously. Subsequently, the same photoresist was applied, exposure development was carried out, and the electrode with the opening 13 to which a conductor 11 is exposed was formed. Under the present circumstances, the configuration of opening 13 was made circular and the electrode which is two kinds whose diameters are 10mm

and 0.1mm was produced.
[0098] Subsequently, the nucleic acid (probe) for detection of beta type hepatitis virus (etaBV) was fixed in these electrodes (1012 copy/cm2 and 1012 copy/cm2). Fixation of a probe and detection of a nucleic acid were carried out by the method shown in the gestalt of operation. And detection sensitivity and the amount of samples required for measurement were measured about each electrode. As shown in Table 3, while detection sensitivity became high by the electrode with a small area of opening (fixed field of a nucleic acid) as a result of the detection experiment, it was able to measure with the sample of a fewer amount.
[0099]
[Table 3]

[Table 3]

開口部の直径 (mm)	検出限界 (copy/mL)	測定に要するサンプルの量 (μl)
10	10^6	200
0.1	10^5	10

(Example 12) In this example, the electrode corresponding to the gestalt of operation shown in drawing 4 was created.
[0100] 7. After the sulfuric acid and the hydrogen-peroxide solution washed the Pyrex-glass substrate 12 of 62 cm (3 inches), by the electron beam method, vacuum evaporationo was carried out so that it might become the thickness of 500A about titanium by the conventional method first, and subsequently gold was similarly deposited so that it might become the thickness of 5000A. Next, lithography was performed using the photoresist AZ4620 for optical exposure, and 2-set (A and B) production of the group which consists of two openings 31 (the diameter of 0.5mm and 2x10 to 3 cm area 2) and opening 32 (the diameter of 0.05mm and 2x10 to 5 cm area 2) from which area differs on one electrode was carried out.
[0101] Subsequently, it fixed in this electrode by the method of showing the nucleic acid (probe) for detection of beta type hepatitis virus (etaBV) in the gestalt of operation (opening 31 and opening 32 1012 copy/cm2). Fixation of a probe and detection of a nucleic acid were carried out by the method shown in the gestalt of operation. In addition, in the A side, it is the subtype of hepatitis B virus. About the probe which detects adr, it is the subtype of hepatitis B virus in the B side. The probe of 20 complementary bases was fixed in ayr. The nucleic acid which originates in hepatitis B virus from the blood serum of a carrier was extracted like the above-mentioned example. After performing rhoCR by the common primer between subtypes to the extracted nucleic acid, stage dilution was carried out and hybridization was performed like the above. After hybridization, after washing the electrode and flooding with the solution of the intercalating-agent HOECHST 33258, the electrochemical signal originating in the HOECHST 33258 was measured. The positive signal was acquired only from opening which fixed the complementary probe in adr the result of a detection experiment. Therefore, it turns out that they are hepatitis B virus contained in the sample, and adr. Moreover, it was able to measure by the dynamic range of 6 figures by using the electrode from which the area of opening differs in the

detection experiment using the sample which carried out stage dilution.
[0102] (Example 13) After the sulfuric acid and the hydrogen-peroxide solution washed the 7.62cm (3 inches) Pyrex-glass substrate, by sputtering, titanium was formed so that it might become the thickness of 500A, and subsequently gold was similarly formed so that it might become the thickness of 5000A. Next, lithography was performed using the photoresist AZ4620 for optical exposure, and the electrode equipped with two openings (the diameter of 0.5mm, the area 2x10-3cm2; diameter of 0.05mm, and 2x10 to 5 cm area 2) from which area differs on one electrode was produced. Subsequently, it fixed in the conductor exposed from opening of this electrode by the method of showing the nucleic acid (probe) for detection of beta type hepatitis virus (etaBV) in the gestalt of operation (two openings 1012 copy/cm2).
[0103] And the blood serum was extracted from the carrier and the nucleic acid which originates in etaBV using the phenol-chloroform method was extracted. It dissolved in the solution of the sodium chloride of 150mM, and the sodium citrate (pH 7.0) of 15mM, and the extracted nucleic acid was used for hybridization. After hybridization, the electrode was washed, it flooded with the solution of intercalating-agent Hoechst 33258, the fluorescence originating in Hoechst 33258 was measured, and detection sensitivity was measured. Consequently, although the difference was not accepted in a S/N ratio among both, in the field of opening with a diameter of 0.05mm, S/N of fluorescence intensity improved with the passage of time, and, 24 hours after, it improved in about 1 hour by about 3 times the S/N in the field of opening with a diameter of 0.5mm. Moreover, detection sensitivity is 5x106 in the field of opening with a diameter of 0.5mm. Although copy/mL was a limitation, in the field of 0.05mm opening, it is 106 then. copy/mL was a limitation. As mentioned above, when an optical signal is detected, it turns out that the one of detection sensitivity where the area of the field which fixes a probe is smaller is high. (Example 14) In this example, the sensor equipped with the electrode corresponding to the form of operation shown in drawing 11 was created.
[0104] After the sulfuric acid and the hydrogen-peroxide solution washed the 2x2cm Pyrex-glass substrates 91 and 92, the electrode 94 and electrode 93 which consist of gold with a diameter of 2mm on this Pyrex-glass substrate 91 and 92 were produced by sputtering. And in the electrode 93, it was fixed between the subtypes of HBV, having used as the probe 95 the nucleic acid which consists of a common array (thiol indicator-ACTTCTCTCAATTTTCTAGG) (1012 copy/cm2), and it was fixed, having used as the probe 96 the nucleic acid which becomes an electrode 94 from another consensus sequence (thiol indicator-CGTCCCGTCGGCGCTGAATC) (1012 copy/cm2). in addition, the area of the field which fixed the probe in the electrode 93 and the electrode 94 -- ten to 2 cm2 it is . Next, the spacer 112 with a thickness of 2000A was inserted between the electrode 93 and the electrode 94, and the distance between an electrode 93 and an electrode 94 was fixed to regularity (2000A). Moreover, fixation of a probe 95 and a probe 96 and detection of a nucleic acid were carried out by the method shown in the form of operation. First, the blood serum was extracted from the carrier and the nucleic acid which originates in etaBV using the phenol-chloroform method was extracted. It dissolved in the solution of the sodium chloride of 150mM, and the sodium citrate (pH 7.0) of 15mM, and the extracted nucleic acid was made into the sample. Next, after passing 1000micro of these samples that carried out thermal denaturation by the flow rate of l/hr and performing hybridization of 1 hour between an electrode 93 and an electrode 94, the electrode 93 and the electrode 94 were fully washed by ultrapure water. Subsequently, between an electrode 93 and electrodes 94 was filled with ultrapure water, electrical conductivity was measured, and HBV was detected. In addition, detection of HBV was performed using two or more samples which

carried out stage dilution. Consequently, it was confirmed that the electrical conductivity between an electrode 93 and an electrode 94 and the concentration of DNA of HBV are in proportionality mostly. Therefore, it was checked that the fixed quantity of the concentration of DNA of HBV can be carried out by measurement of inter-electrode electrical conductivity.
[0105] (Example 15) After the sulfuric acid and the hydrogen-peroxide solution washed the Pyrex-glass substrate (2x4cm and 2x2cm) 101, and 102 and 103, the electrodes 104, 105, and 106 which consist of gold with a diameter of 2mm on these Pyrex-glass substrates 101 and 102 and 103 were produced by sputtering. And in the electrode 104, it was fixed between the subtypes of HBV, having used as the probe 107 the nucleic acid which consists of a common array (thiol indicator-ACTTCTCTCAATTTTCTAGG) (1012 copy/cm2). On the other hand, in an electrode 105, it is the subtype of HBV. An alternative array (thiol indicator-CGTCCCGTCGGCGCTGAATC) is fixed in ayr (1012 copy/cm2), and it is the subtype of HBV in an electrode 106. The alternative array (thiol indicator-CGTCCCGTCGGCGCTGAATC) was fixed in adw (1012 copy/cm2), and the sensor equipped with composition as shown in drawing 10 was created. Next, the spacer of thickness 2000A was inserted between the electrode 104 and the electrode 105, the electrode 104, and the electrode 106, and the distance between an electrode 104 and an electrode 105, an electrode 104, and an electrode 106 was fixed to regularity (2000A). Moreover, fixation of a probe 95 and a probe 96 and detection of a nucleic acid were carried out by the method shown in the form of operation. Next, thermal denaturation of the sample obtained like the example 14 between the electrode 104 and the electrode 105, the electrode 104, and the electrode 106 was carried out, 1000micro was circulated by the flow rate of l/hr, and hybridization of 1 hour was performed. in addition, it was made to each every 30 just electrify an electrode 104 and electrode 105 ****** 106 through the process of hybridization And the electrode 104, the electrode 105, and the electrode 106 were fully washed by ultrapure water. Subsequently, between an electrode 104 and an electrode 105, an electrode 104, and electrodes 106 was filled with ultrapure water, electrical conductivity was measured, and HBV was detected. Consequently, although electrical conductivity fell between the electrode 104 and the electrode 105, between an electrode 104 and an electrode 106, there was no change in electrical conductivity. Therefore, it was checked that they are HBV contained in the above-mentioned sample and an ayr type.
[0106] (Example 16) In this example, the sensor equipped with the electrode corresponding to the form of operation shown in drawing 11 was created.
[0107] After the sulfuric acid and the hydrogen-peroxide solution washed the 2x2cm Pyrex-glass substrates 91 and 92, the electrodes 94 and 93 which consist of gold with a diameter of 2mm on this Pyrex-glass substrate 91 and 92 were produced by sputtering. And in the electrode 93, it was fixed between the subtypes of HBV, having used as the probe 95 the nucleic acid which consists of a common array (thiol indicator-ACTTCTCTCAATTTTCTAGG) (1012 copy/cm2), and it was fixed, having used as the probe 96 the nucleic acid which becomes an electrode 94 from another consensus sequence (thiol indicator-CGTCCCGTCGGCGCTGAATC) (1012 copy/cm2). in addition, the area of the field which fixed the probe in the electrode 93 and the electrode 94 -- ten to 2 cm2 it is . Next, the spacer 112 with a thickness of 2000A was inserted between the electrode 93 and the electrode 94, and the distance between an electrode 93 and an electrode 94 was fixed to regularity (2000A). Moreover, fixation of a probe 95 and a probe 96 and detection of a nucleic acid were carried out by the method shown in the form of operation.
[0108] Next, it is L/hr 1000micro about the probe 111 which carried out the indicator by this sample and lucigenin which carried out thermal denaturation between the electrode 93 and the

electrode 94. After passing by the flow rate and performing hybridization of 1 hour, the electrode 93 and the electrode 94 were fully washed by ultrapure water. And the luminescence intensity which impressed voltage and was generated between the electrode 93 and the electrode 94 was measured, and hepatitis B virus was detected. In addition, detection of hepatitis B virus was performed using two or more samples which carried out stage dilution. Consequently, it was confirmed that the measured luminescence intensity and the concentration of DNA of hepatitis B virus are in proportionality mostly. Therefore, it was checked that the fixed quantity of the concentration of DNA of hepatitis B virus can be carried out by measurement of luminescence intensity.

[0109] (Example 17) In this example, the sensor equipped with the electrode corresponding to the gestalt of operation shown in drawing 11 was created.

[0110] After the sulfuric acid and the hydrogen-peroxide solution washed the 2x2cm Pyrex-glass substrates 91 and 92, the electrode 94 and electrode 93 which consist of gold with a diameter of 2mm on this Pyrex-glass substrate 91 and 92 were produced by sputtering. And in the electrode 93, it was fixed between the subtypes of hepatitis B virus, having used as the probe 95 the nucleic acid which consists of a common array (thiol indicator-ACTTCTCTCAATTTTCTAGG) (1012 copy/cm2), and it was fixed, having used as the probe 96 the nucleic acid which becomes an electrode 94 from another consensus sequence (thiol indicator-CGTCCCGTCGGCGCTGAATC) (1012 copy/cm2). in addition, the area of the field which fixed the probe in the electrode 93 and the electrode 94 -- ten to 2 cm2 it is . Next, the spacer 112 with a thickness of 2000A was inserted between the electrode 93 and the electrode 94, and the distance between an electrode 93 and an electrode 94 was fixed to regularity (2000A). Moreover, fixation of a probe 95 and a probe 96 and detection of a nucleic acid were carried out by the method shown in the gestalt of operation. Next, it is L/hr 1000micro about the probe 111 which carried out the indicator by the same sample and same FIROSEN as the example 14 which carried out thermal denaturation between the electrode 93 and the electrode 94. After passing by the flow rate and performing hybridization of 1 hour, the electrode 93 and the electrode 94 were fully washed by ultrapure water. Subsequently, while filling between an electrode 93 and electrodes 94 with ultrapure water and impressing voltage, silver/silver silver chloride electrode (reference electrode) was inserted into the ultrapure water between an electrode 93 and an electrode 94, the current value of the ferrocene origin was measured, and hepatitis B virus was detected. Consequently, it was confirmed that the concentration of the current value of the ferrocene origin and DNA of hepatitis B virus obtained on the operation pole (an electrode 93 and electrode 94) is in proportionality mostly. Therefore, it was checked that the fixed quantity of the concentration of DNA of hepatitis B virus can be carried out by measurement of the current value which flows to inter-electrode.

[0111] (Example 18) In this example, the sensor equipped with the electrode corresponding to the form of operation shown in drawing 11 was created.

[0112] After the sulfuric acid and the hydrogen-peroxide solution washed the 2x2cm Pyrex-glass substrates 91 and 92, the electrodes 94 and 93 which consist of carbon with a diameter of 2mm on this Pyrex-glass substrate 91 and 92 were produced by sputtering. And after processing the front face of electrodes 93 and 94 by 3-amino pro pilus triethoxysilane, in the electrode 93, it was fixed between the subtypes of HBV, having used as the probe 95 the nucleic acid which consists of a common array (amino-group-ACTTCTCTCAATTTTCTAGG) (1013 copy/cm2), and it was fixed, having used as the probe 96 the nucleic acid which becomes an electrode 94 from another consensus sequence (amino-group-CGTCCCGTCGGCGCTGAATC) (1013

copy/cm2). in addition, the area of the field which fixed the probe in the electrode 93 and the electrode 94 -- ten to 4 cm2 it is . Next, the spacer 112 with a thickness of 2000A was inserted between the electrode 93 and the electrode 94, and the distance between an electrode 93 and an electrode 94 was fixed to regularity (2000A). Moreover, fixation of a probe 95 and a probe 96 and detection of a nucleic acid were carried out by the method shown in the bottom of coexistence of a glutaraldehyde, and the form of operation.

[0113] Next, after passing 1000micro of the same samples as the example 14 which carried out thermal denaturation by the flow rate of l/hr and performing hybridization of 1 hour between an electrode 93 and an electrode 94, the electrode 93 and the electrode 94 were fully washed by ultrapure water. Subsequently, between an electrode 93 and electrodes 94 was filled with ultrapure water, the platinum electrode (counter electrode), and the silver/silver silver chloride electrode (reference electrode) were inserted into this ultrapure water, and voltage was impressed between the electrode 93 and the electrode 94 (operation pole). And feeble luminescence produced from a nucleic acid was measured, and HBV was detected. Consequently, it was confirmed that the concentration of DNA of feeble luminescence produced from a nucleic acid and HBV is in proportionality mostly. Therefore, it was checked that the fixed quantity of the concentration of DNA of HBV can be carried out by measurement of feeble luminescence produced from a nucleic acid.

[0114] Next, the example which performed detection of a nucleic acid to high sensitivity is explained in full detail, without presenting improvement in the extraction efficiency of a nucleic acid, and false positivity.

[0115] hepatitis-B-virus the non-infected blood serum originating in the panel blood serum and healthy person (person not qualified as a senior official) originating in the patient of hepatitis B respectively (Example 19) To 1mL Proteinase K and a surfactant (0.5% SDS) are made to act. After breaking the coat of a virus and exposing DNA, the convolution-kernel acid (poly A) which becomes each from 5 -100mer was added so that it might be set to 100ng/mL, and DNA was extracted using the FENO 1 RU chloroform method. After carrying out alkali denaturation of the extracted DNA, detection by dot blotting technique was performed using the probe (5'-CGTCCCGTCGGCGCTGAATC-3' complementary to X field of etaBV) which carried out the blot to the nitrocellulose membrane and which carried out the label by RI. Moreover, same operation was performed using DNA which extracted DNA and the carrier nucleic acid which added and extracted the cow thymus gland DNA as control by un-adding. In addition, in the above-mentioned blood serum, the amount of viruses is known and two or more blood serums in which the amount of viruses from low concentration to high concentration is shown were prepared.

[0116] the blood serum in which the high-concentration amount of viruses is shown as a result of operation -- receiving -- the above -- although the spot of equivalent intensity was able to be checked on the film by any method, in DNA which extracted the carrier nucleic acid by un-adding to the blood serum in which the low-concentration amount of viruses is shown, the intensity of a signal was weak Moreover, although a spot was not seen at all when a convolution-kernel acid (poly A) was added and DNA was extracted about HBV a non-infected blood serum, when the cow thymus gland DNA was added and DNA was extracted, the spot was seen weakly, and the distinction with the spot of the blood serum in which the low-concentration amount of viruses is shown did not stick.

[0117] It was confirmed that a nucleic acid with little (low concentration) abundance can also be efficiently extracted from the above thing by adding a carrier nucleic acid. Moreover, when the

cow thymus gland DNA was used as a carrier nucleic acid, the reaction was slightly caused between probes (hybrid formation), and the danger of judging HBV a non-infected blood serum to be a positivity was shown. This is guessed because an analogous base sequence exists in Carrier DNA at HBV. Moreover, when poly A was used as a carrier DNA, it was checked that there is no danger of false positivity.

[0118] (Example 20) The following model experiments were conducted using pYRB 259 which included etaBV-DNA in the plasmid pSP 65, and produced it.

[0119] After making a microtiter plate adsorb a probe (5'-CGTCCCGTCGGCGCTGAATC-3') complementary to X field of etaBV, ultraviolet rays were irradiated and the probe was fixed on the microtiter plate (1012 copy/cm2). On the other hand, pYRB 259 which carried out the label by the biotin as a target was produced. Next, biotin-ized pYRB 259 so that it may be set to 100ng/mL moreover, the synthetic oligomer (5'-ACTTCTCTCAATTTTCTAGG-3' --) which equipped HBV-DNA with the complementary array as a carrier nucleic acid 5'-CGTCGCAG****GATCTCAATC-3', It is a hybridization solution (chlorination NA ** RIUMU 150m mol/L) so that 5'-TCGTGTTACAGGCGGGGTTT-3' and 5'-CGAACCACTGAACAAATGGC-3' may be respectively set to 1ng/mL. After dissolving and carrying out thermal denaturation to the sodium citrate of 15m mol/L, and pH 7.0, it put into the well of the plate which fixed the probe every [100micro / l], and hybridization was performed at 43 degrees C for 1 hour.

[0120] next, the amount of pYRB 259 in which the probe and hybrid on the amount of pYRB 259 which remains in reaction mixture after performing hybridization, and a plate were formed -- a rabbit anti-biotin antibody -- and -- HRP indicator anti-rabbit It measured in immunochemistry using the IgG antibody. Moreover, same operation was performed also with the case where synthetic oligomer is not added, as control.

[0121] Consequently, when synthetic oligomer was added as shown in Table 4, the amount of the target (pYRB 259) which remains in about 2.6 times and reaction mixture as compared with the control whose amount of the target (pYRB 259) in which the probe and hybrid on a microtiter plate were formed had not added synthetic oligomer was about 1/3 as compared with the control which had not added synthetic oligomer.

[0122]

[Table 4]

	プレートに固定化されたプローブとハイブリダイズしたターゲットの量 (A_{415})	ハイブリダイゼーション溶液に残存したターゲットの量 (A_{415})
オリゴマーを添加した場合	1.86	0.53
オリゴマーが無添加の場合	0.70	1.62

As mentioned above, it was checked that the efficiency in which own self hybridization of a target is suppressed by addition of a carrier nucleic acid, and a target forms the probe and hybrid on a microtiter plate by it improves.

[0123] (Example 21) A AT cut, the oscillation frequency of 9MHz, and an electrode area of 0.196cm, 2 and the electrode material fixed complementary 5'-CGTCCCGTCGGCGCTGAATC-3' through the thiol group to X field of etaBV in the quartz resonator which is gold, and produced etaBV-DNA detection sensor. After having dissolved the ultrasonic decomposition product of a plasmid pSP 65 in the hybridization solution (chlorination NA ** RIUMU of 150m mol/L, the sodium citrate of 15m mol/L, pH 7.0) so that it might be set to 100ng/mL, respectively, and carrying out thermal denaturation of the 5mL(s) of this solution as pYRB 259 and a carrier nucleic acid as a target, the above-mentioned quartz resonator was inserted in this solution, and hybridization was performed at 43 degrees C for 1 hour. On the other hand, operation with the same said of the case where the ultrasonic decomposition product of pSP 65 is not added, as control was performed.

[0124] Consequently, although the decrement of the vibration frequency of a quartz resonator was about 100Hz when the ultrasonic decomposition product of pSP 65 was not added, in the case where the ultrasonic decomposition product of pSP 65 is added, the decrement of the vibration frequency of a quartz resonator was about 350Hz. Since the ultrasonic decomposition product of pSP 65 hybridized this result further to pYRB 259 in which the probe and hybrid on a quartz resonator were formed, it is shown that the variation of the vibration frequency of a quartz resonator increased, and it was checked that a detecting signal increases by addition of a carrier nucleic acid.

[0125] (Example 22) In this example, the electrode corresponding to the form of operation shown in drawing 2 was manufactured.

[0126] 7. After the sulfuric acid and the hydrogen-peroxide solution washed the Pyrex (registered trademark) glass substrate 12 of 62 cm (3 inches), by sputtering, membranes were formed so that it might become the thickness of 500A about titanium by the conventional method first, and subsequently gold was similarly formed so that it might become the thickness of 5000A. Next, lithography of a vacuum evaporationo metal was performed using the photoresist AZ4620 for optical exposure, and the conductor 11 was formed. Subsequently, the same photoresist was applied, exposure development was carried out, and the electrode with the opening 13 (area : 0.07cm 2) to which a conductor 11 is exposed was formed.

[0127] Subsequently, the nucleic acid (5'-CGTCCCGTCGGCGCTGAATC-3') complementary to X field of beta type hepatitis virus (etaBV) as a probe was fixed through the thiol group in this electrode (1012 copy/cm2).

[0128] Next, it is a hybridization solution (chlorination NA ** RIUMU 150m mol/L) so that the ultrasonic decomposition product of a plasmid pSP 65 may be set to 103 copy/mL as pYRB 259 and a carrier nucleic acid as a target, respectively. It dissolves in the sodium citrate of 15m mol/L, and pH 7.0, and is 100microL of this solution. After heating and carrying out thermal denaturation of the DNA, the above-mentioned electrode was put in into this solution, and high buri tie ZESHON was performed at 43 degrees C for 1 hour. And electrochemical measurement was performed after having washed the electrode after performing hybridization, and flooding with the solution of KISUTO 33258 to an intercalating agent. On the other hand, it was operated like the above-mentioned case as control except not adding the ultrasonic decomposition product of pSP 65.

[0129] Consequently, when the current value originating in Hoechst 33258 added the ultrasonic

decomposition product of pSP 65, the significant difference of 5nA(s) was accepted as compared with the control which does not add the ultrasonic decomposition product of pSP 65. Since the ultrasonic decomposition product of pSP 65 hybridized this result further to pYRB 259 hybridized to the probe on an electrode, it is shown that the amount of combination of Hoechst 33258 increased, and it was checked that a detecting signal increases by addition of a carrier nucleic acid.

[0130] (Example 23) In this example, the electrode corresponding to the form of operation shown in drawing 2 was manufactured.

[0131] 7. After the sulfuric acid and the hydrogen-peroxide solution washed the Pyrex (registered trademark) glass substrate 12 of 62 cm (3 inches), by the electron beam method, vacuum evaporationo was carried out so that it might become the thickness of 500A about titanium by the conventional method first, and subsequently gold was similarly deposited so that it might become the thickness of 5000A. Next, lithography was performed using the photoresist AZ4620 for optical exposure, and the glue line 15 and the conductor 11 were formed simultaneously. Furthermore, membranes were formed so that it might become the thickness of 2000A about a silicon nitride film by CVD, and the insulator 14 was made to form. Subsequently, the same photoresist was applied, exposure development was carried out, and the electrode equipped with the resist pattern (insulator) with the opening 13 (area : 0.07cm 2) to which a conductor is exposed was formed.

[0132] Subsequently, the nucleic acid which has complementary base sequence 5'-CGTCCCGTCGGCGCTGAATC-3' in X field of etaBV as a probe was fixed through the thiol group on the conductor exposed to the opening 13 of this electrode.

[0133] Blood serum 500microL which originates in the patient of hepatitis B on the other hand After processing with Proteinase kappa and a surfactant (0.5% SDS), the synthetic oligomer (5'-ACTTCTCTCAATTTTCTAGG-3' --) which is the convolution-kernel acid (poly A) which consists of 5 -100mer as a carrier nucleic acid, and a base sequence complementary to etaBV 5'-CGTCGCAGAAGATCTCAATC-3', 5'-TCGTGTTACAGGCGGGGTTT-3', and 5'-CGAACCACTGAACAAATGGC-3' are added so that it may be respectively set to 0.1ng/mL. DNA was extracted using the FENO 1 RU chloroform method. And extracted DNA is dissolved in hybridization solution (chlorination NA ** RIUMU [of 150m mol/L], sodium-citrate [of 15m mol/L], pH 7.0) 500microl, and it is this solution. After heating 100microl and carrying out thermal denaturation of the DNA, the above-mentioned electrode was put in into the solution, and high buri tie ZESHON was performed at 43 degrees C for 1 hour. And electrochemical measurement was performed after having washed the electrode after performing hybridization, and flooding with the solution of KISUTO 33258 to an intercalating agent. Furthermore, it was operated like the above-mentioned case as control except not adding a carrier nucleic acid. On the other hand, as a sample for calibration curves, HBV-DNA of known concentration was diluted with the blood serum extract (what passed through the same extract operation by the normal people blood serum), and it measured similarly using what added the above-mentioned carrier nucleic acid.

[0134] Consequently, since the proportional region of a calibration curve was 105-107 copy/mL in the control which did not add a carrier nucleic acid as shown in B of drawing 16 , although the sample which separates from the upper limit or minimum of a calibration curve existed mostly Since the proportional region of a calibration curve spread with 103-108copy/mL as shown in A of drawing 16 when a carrier nucleic acid was added, the range (copy number) which can detect HBV-DNA was expanded, and the sample which shifts from this range decreased remarkably.

Therefore, it was checked by adding a carrier nucleic acid that the remarkable improvement in limit of detection is attained. In addition, by processing by modifiers, such as hot water processing of 90 degrees C or more, alkali solution processing 0.2 mols / beyond L grade, or a urea, it can reproduce easily and all the electrodes mentioned above can be used repeatedly.
[0135]

EFFECT OF THE INVENTION

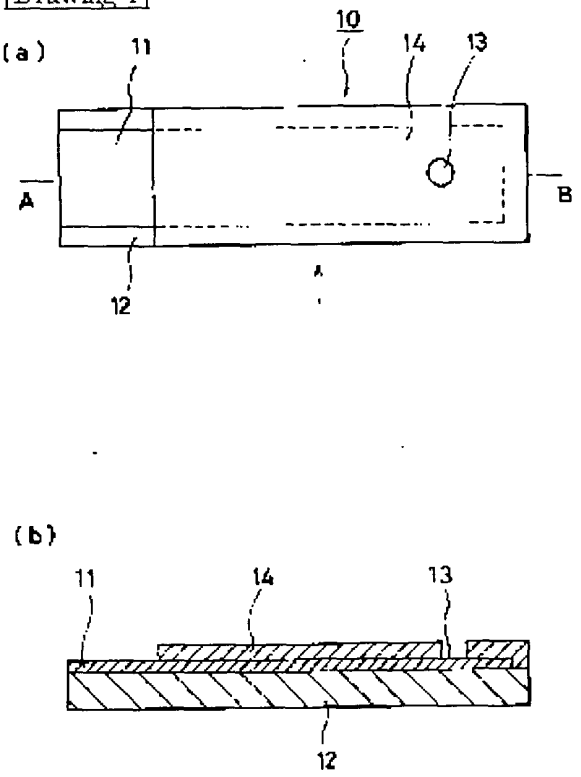
According to the electrode built over this application the 1st invention as explained in full detail above, a nucleic acid is fixable to up to the conductor by which the surface area was controlled by having prepared opening so that a conductor might be exposed to some insulators which covered the conductor arranged on a substrate, and having fixed the nucleic acid in the conductor exposed from this opening. Moreover, the property of a conductor is controllable by having arranged the conductor on a substrate. The surface area and crystal **** of an electrode which fix a probe are controllable. Therefore, the outstanding repeatability and outstanding fixed quantity nature can be demonstrated, and the electrode which can detect a nucleic acid can be offered economically.
[0136] Moreover, according to the detection equipment concerning this application the 2nd invention, hybridization of the 1st nucleic acid and 2nd nucleic acid which were fixed quantitatively is carried out to an electrode in the reaction section. Shortening of time and the improvement in operability which the measurand of this signal and the amount of the 2nd nucleic acid can be made to correspond uniquely, and detection takes are attained by measuring the signal which impressed voltage to the 1st nucleic acid and was produced. Therefore, while demonstrating the outstanding repeatability and outstanding fixed quantity nature on the occasion of detection of a nucleic acid, the detection equipment which is excellent also in the economical efficiency at the time of the detection by which shortening of time and the improvement in operability which detection takes were achieved can be offered.
[0137] Furthermore, according to the detection equipment concerning this application the 3rd invention, hybridization of the 1st nucleic acid and 2nd nucleic acid which were fixed quantitatively is carried out in the reaction section into the field which has the minimum area required for detection of the 2nd nucleic acid. By measuring the signal which impressed voltage to the 1st nucleic acid and was produced, while making the measurand of this signal, and the amount of the 2nd nucleic acid correspond uniquely, the background can be reduced, and shortening of time and the improvement in operability which detection takes can be attained. Therefore, while demonstrating the outstanding repeatability and outstanding fixed quantity nature on the occasion of detection of a nucleic acid, high sensitivity-ization of detection sensitivity is attained and the detection equipment which is excellent also in the economical efficiency at the time of the detection by which shortening of time and the improvement in operability which detection takes were achieved can be offered.
[0138] It arranges so that the 3rd nucleic acid can form a hybrid to the 1st and the 2nd nucleic acid of the above. moreover, the 1st and 2nd electrodes which fixed the 1st and 2nd nucleic acids according to the detection equipment concerning this application the 4th invention -- this -- By measuring the signal which impressed voltage and was produced from the power supply connected to the 1st and 2nd electrodes, the signal originating in the 3rd nucleic acid can be acquired certainly, reducing the background. Therefore, while demonstrating the outstanding repeatability and outstanding fixed quantity nature on the occasion of detection of a nucleic acid,

high sensitivity-ization of detection sensitivity is attained and the detection equipment which is excellent also in the economical efficiency at the time of the detection by which shortening of time and the improvement in operability which detection takes were achieved can be offered.
[0139] Moreover, according to the detection equipment concerning this application the 5th invention, the 3rd nucleic acid can form easily the hybrid stabilized to the 1st and 2nd nucleic acids by having arranged the 1st and 2nd electrodes which fixed the 1st and 2nd nucleic acids so that the 3rd nucleic acid can form a hybrid to the 1st and 2nd nucleic acids. Therefore, while demonstrating the outstanding repeatability and outstanding fixed quantity nature on the occasion of detection of a nucleic acid, high sensitivity-ization of detection sensitivity is attained and the sensor which is excellent also in the economical efficiency at the time of the detection by which shortening of time and the improvement in operability which detection takes were achieved can be offered. the drawings, any words are not translated.

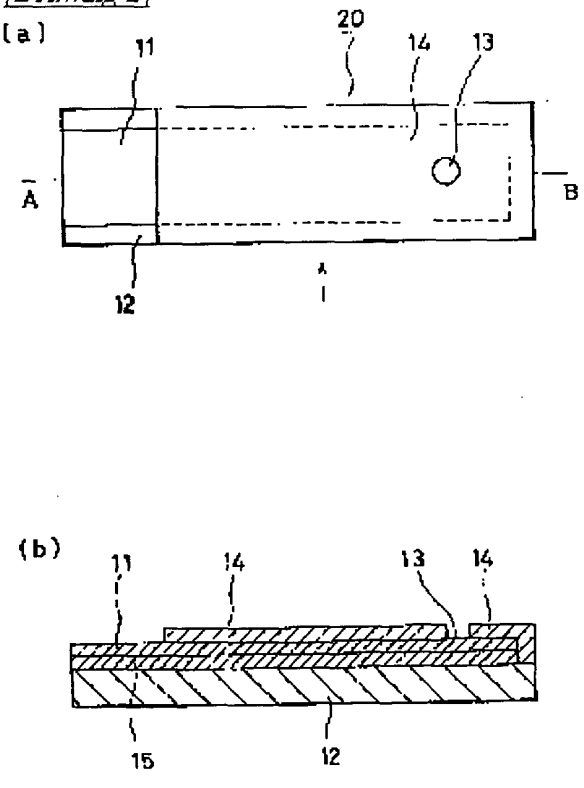
CLAIMS

[Claim(s)]
[Claim 1] The electrode characterized by providing a substrate, the conductor arranged on the aforementioned substrate, the insulator covered securing a connection field [as opposed to the exterior for the front face of the aforementioned conductor], opening prepared in the aforementioned insulator so that the aforementioned conductor might be exposed, and the nucleic acid fixed in the aforementioned conductor exposed from the aforementioned opening.
[Claim 2] Detection equipment characterized by providing the following. Substrate. The conductor arranged on the aforementioned substrate. The insulator covered securing a connection field [as opposed to the exterior for the front face of the aforementioned conductor]. The electrode which has opening prepared in the aforementioned insulator so that the aforementioned conductor might be exposed, and the 1st nucleic acid fixed in the aforementioned conductor exposed from the aforementioned opening, The reaction section which performs hybridization under the conditions which the 1st nucleic acid and 2nd nucleic acid which were fixed by the aforementioned electrode were made to live together, and were set up, an impression means to impress voltage to the 1st nucleic acid fixed by the aforementioned electrode, and a measurement means to measure the signal produced by operation of the aforementioned impression means.
[Claim 3] Detection equipment characterized by providing the following. The electrode which fixed the 1st nucleic acid in the field which has the minimum area required for detection of the 2nd nucleic acid. The reaction section which performs hybridization under the conditions which the 1st nucleic acid and 2nd nucleic acid of the above which were fixed by the aforementioned electrode were made to live together, and were set up. An impression means to impress voltage to the 1st nucleic acid fixed by the aforementioned electrode. A measurement means to measure the signal produced by operation of the aforementioned impression means.
[Claim 4] Detection equipment characterized by providing the following. The 1st electrode which fixed the 1st nucleic acid. The 2nd electrode arranged so that the 3rd nucleic acid can form a hybrid to the above 1st and the 2nd nucleic acid of the above while fixing the 2nd nucleic acid. The power supply connected to the above 1st and the 2nd electrode of the above. A measurement means to measure the signal produced by operation of the aforementioned power supply.
[Claim 5] The sensor characterized by providing the 1st electrode which fixed the 1st nucleic acid, and the 2nd electrode arranged so that the 3rd nucleic acid can form a hybrid to the above 1st and the 2nd nucleic acid of the above while fixing the 2nd nucleic acid.

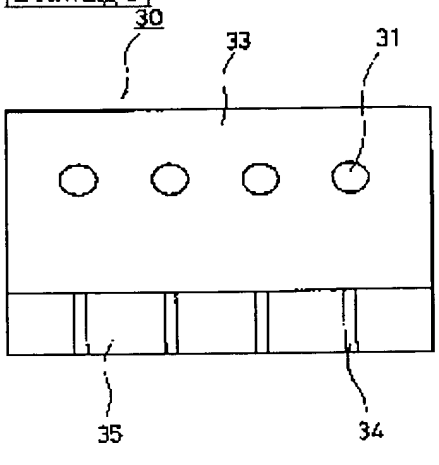
[Drawing 1]



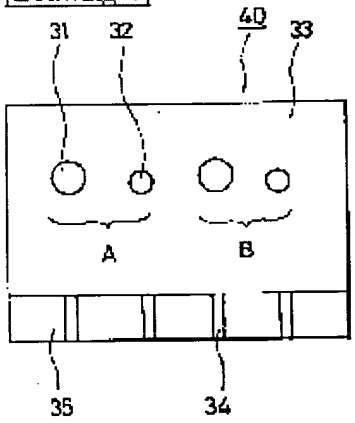
[Drawing 2]



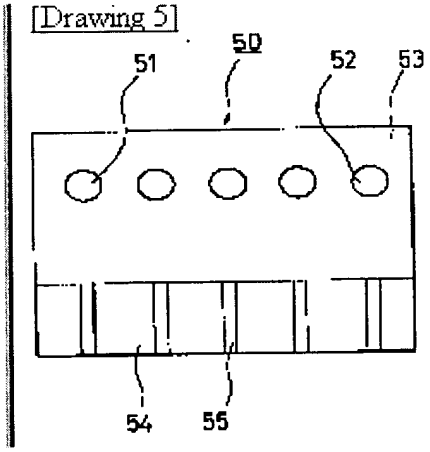
[Drawing 3]



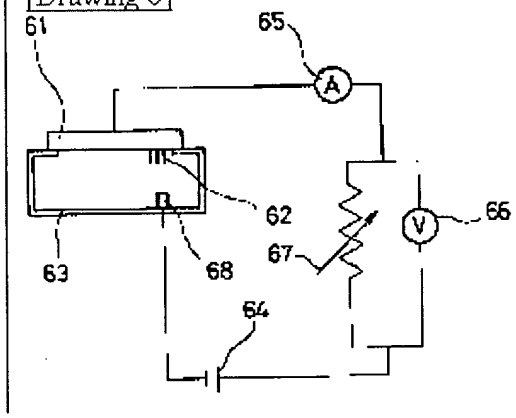
[Drawing 4]



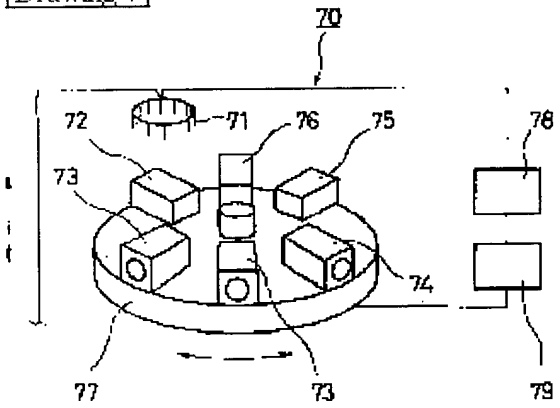
[Drawing 5]



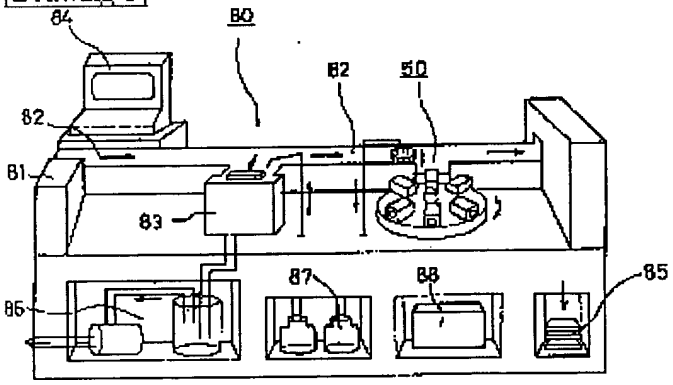
[Drawing 6]



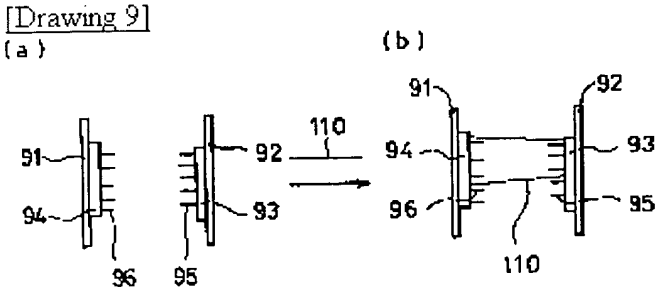
[Drawing 7]



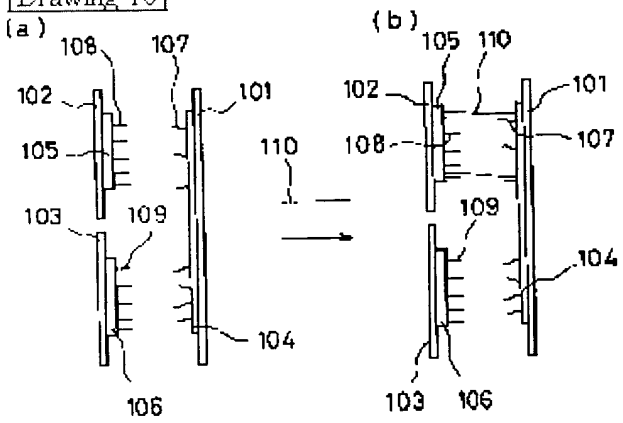
[Drawing 8]



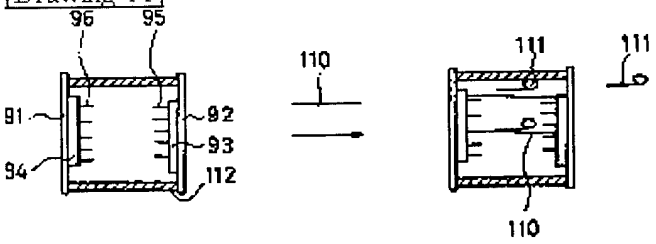
[Drawing 9]



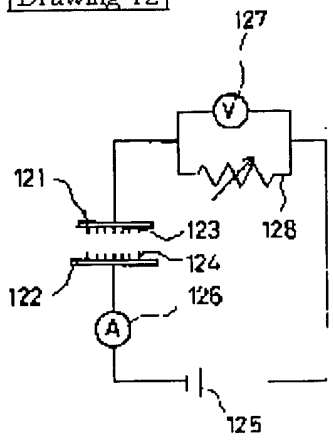
[Drawing 10]



[Drawing 11]

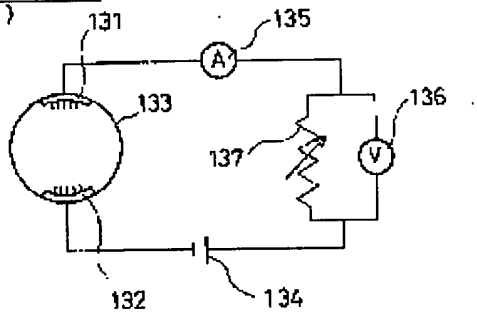


[Drawing 12]

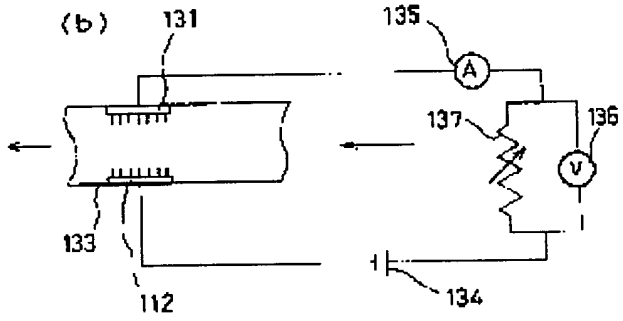


[Drawing 13]

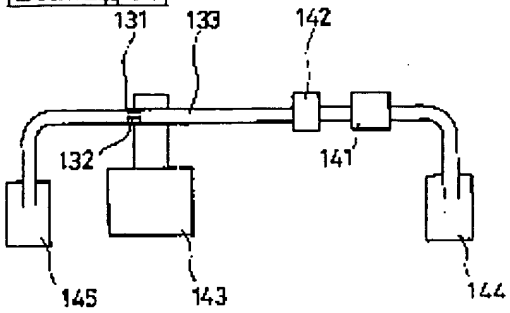
(a)



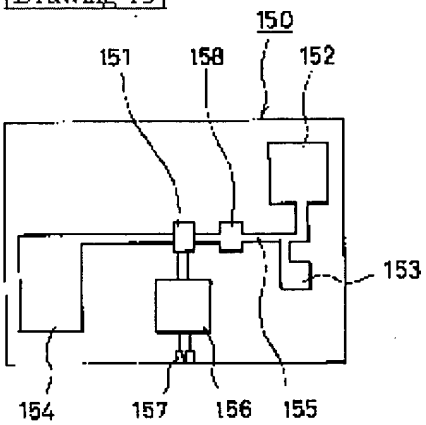
(b)



[Drawing 14]



[Drawing 15]



[Drawing 16]